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Sustainable Management of Duckweed Biomass Grown for Nutrient Control in Municipal Wastewaters

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SUSTAINABLE MANAGEMENT OF DUCKWEED BIOMASS GROWN FOR NUTRIENT CONTROL IN MUNICIPAL WASTEWATERS

by

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ABSTRACT

Sustainable Management of Duckweed Biomass Grown for Nutrient Control in Municipal Wastewaters

by

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The use of duckweed as a nutrient removal option for municipal wastewaters can only be realized through regular plant harvesting. As a result, the nutrient-rich biomass generated needs to be effectively managed and disposed of. This study looked at three alternative options for biomass management that would make duckweed-based nutrient removal systems sustainable and attractive to small communities like Wellsville City, Utah. The options included: the use of harvested duckweed biomass as an animal feed, anaerobic digestion of duckweed for methane production, and fermentation of biomass for ethanol production.

Duckweed feed quality was determined using feed analysis reports and results from digestibility studies (in vitro fermentation). The performance of the anaerobic digestion process was determined by monitoring pH, VS, TS, NH_4-N, VFAs, and

alkalinity. The ethanol production yields were obtained from starch content values and ethanol concentrations observed from batch fermentation experiments.

Duckweed was composed of 21- 38% crude protein, 94 – 96% water, 78.5% organic matter, < 10% starch and an average of 19% starch after accumulation by nutrient starvation. Relative feed values (RFVs) of 230 – 241, crude protein content of 21-38%, and neutral and acid detergent fiber values of 30.2% and 13.7%, respectively, showed duckweed as a potential feed for ruminants comparable to alfalfa and corn silage (RFVs of 100). Digester performance showed an average methane yield of 6.3 and 5.8 ft^3/lb VS destroyed with methane composition values of 67.1% and 62.5% for fresh DW fed reactor (R1) and air dried DW fed reactor (R2), respectively. The ethanol production yield observed was less than 100 mg ethanol/g DW for both fresh and oven dried DW samples. The recommended duckweed biomass management option for a small community like Wellsville is anaerobic digestion because it is a source of energy and at the same time the digestate can be used as a low-quality feed.

(96 pages)

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Maureen Kesaano

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INTRODUCTION

Background and Justification of the Study

Water quality conditions in the Little Bear River do not meet the standards set by the State of Utah for its Class 3A designated use according to the UDEQ (2009). The pollutants of main concern are total phosphorus and total suspended solids, both below and above the Hyrum Reservoir. The increase in total phosphorus loading below the Hyrum Reservoir is partly attributed to the Wellsville City sewage treatment lagoons (UDEQ, 2009). The UPDES permit for the lagoons limits the discharge of total phosphorous into the Little Bear River to 72 kg/season (June – September) and 360 kg/season (October – May) as a measure enforced by the State to improve receiving water quality.

The Wellsville City sewage treatment lagoons are facultative with a primary focus on Biochemical Oxygen Demand (BOD) and Total Suspended Solids (TSS) removal. Raw wastewater is allowed to settle and microorganisms consume the organic matter during cell synthesis thereby reducing the overall BOD and TSS. Consequently the presence of nutrients in the water and sunlight promote the growth of aquatic plants in the upper aerobic zone of the lagoons. These aquatic plants are known as duckweed. They are small, green, freshwater plants with leaf-like fronds and short roots in the *Lemnaceae* family. They have high reproduction rates and tolerance to cold temperatures (Reed, Crites, and Middlebrooks, 1995). The growing plants form a floating mat on the surface of the water and this surface cover inhibits algae growth, stabilizes pH, and enhances sedimentation (Zirschky and Reed, 1988). The plant density on the water surface depends

on the availability of nutrients, temperature conditions and the frequency of harvest. Studies show the typical plant density on wastewater ponds ranges from 1.2 kg/m^2 to 3.6 kg/m^2 wet weight (0.25 - 0.75 lb/ft^2) (Reed, Crites, and Middlebrooks, 1995) with a typical growth rate of about 0.49 $kg/m^2/d$ (0.1 $lb/ft^2/d$).

The presence of duckweed on the lagoons can be taken advantage of in addressing the water quality concerns of the Little Bear River. By incorporating the duckweed into the facility's wastewater treatment system, its nutrient removal capabilities can be utilized and thus significant improvement to the effluent water quality will be realized. Nutrient removal by the duckweed is through plant uptake (metabolism and bioaccumulation) and subsequent removal from the system by harvesting of the plant biomass. Currently, duckweed growing on the Wellsville lagoons dies off and decomposes at the bottom of the lagoon, forming benthic sludge and releasing much of the nutrients back into the water. This greatly undermines nutrient removal through the treatment process.

Since the plant's physical form facilitates regular harvesting, and the duckweed's rapid growth rate contributes to significant biomass production (Kitani and Hall, 1989), a holistic approach should be adopted that utilizes a duckweed based treatment system to remove phosphorus (nutrients) from the municipal wastewater and at the same time provide valuable by-products for the community. The challenge therefore lies in proper management, alternate re-use options and subsequent disposal of the biomass generated. This research therefore evaluates the feasibility of anaerobic digestion, fermentation for ethanol production and animal feed re-use options for the harvested duckweed biomass.

Problem Statement

In order to effectively implement duckweed systems for nutrient removal in municipal wastewaters, proper management, handling and disposal of harvested biomass is essential. Large quantities of biomass harvested may not only contain undesirable contaminants that may negatively impact the environment but also contain large amounts of water that may not be desirable for landfilling or incineration. There is therefore a need to find ways to utilize the biomass generated in order to make these systems more productive and economically feasible for the community.

Study Objectives

The aim of this research is to evaluate the various options for duckweed biomass processing and disposal/reuse techniques and determine which option(s) is/(are) suitable for small communities like Wellsville City.

The specific objectives of the study are to determine the;

i. Characteristics of the harvested duckweed in terms of nutrients, mineral content and starch content,
ii. Effectiveness of solids processing and reuse options (anaerobic digestion, animal feed, and ethanol production) and corresponding pre-processing requirements for this harvested duckweed
iii. Recommended alternative duckweed biomass management option(s) to optimize cost efficient nutrient management through the Wellsville City lagoons.

Site Description

This research focused on duckweed plants grown on the Wellsville City lagoons found in Cache Valley, Utah (Figure 1). The 56.6-acre lagoons are located in a valley sheltered from the wind by the hills and trees found along the portion of the Little Bear River that flows besides the lagoons (Figure 1). This site provides ideal growing conditions for the duckweed due to the abundance of nutrients from the sewage discharge and shelter from the wind. The growth period in the lagoons spans from late spring to early winter months (early May – early November).

Figure 1. Wellsville City sewage lagoons adapted from Google maps.

LITERATURE REVIEW

Interest in duckweed has been driven by the realization that the plants could be utilized in a number of ways such as: uptake of nutrients and mineral contaminants from wastewater effluent (Körner, Vermaat, and Veenstra, 2003; El-Shafai et al., 2006; Zirschky and Reed, 1988), animal feed (Cheng and Stomp, 2009; Zirschky and Reed, 1988; Skillicorn, Spira, and Journey, 1993), compost (Iqbal, 1999), and bio-energy production (Fedler et al., 2007; Cheng and Stomp, 2009). The ensuing chapter discusses some of the findings from studies carried out on the different species of duckweed located on all continents.

The Duckweed

Duckweed are fast growing aquatic macrophyte plants that float on the surface of stagnant or slow moving water bodies (Skillicorn, Spira, and Journey, 1993). They are classified under the *Lemnaceae* family which consists of about 40 species in five genera; *Spirodela*, *Lemna*, *Landolita*, *Wolffiella* and *Wolffia* (Skillicorn, Spira, and Journey, 1993; Lyerly, 2004; Michael et al., 2008). The species found on the Wellsville Sewage lagoons are *Lemna minor* and *Wolffia*. These two species coexist on the lagoons although *Lemna minor* appears to be the more dominant species. The species are easily differentiated by size, i.e., the fronds of *Lemna* species typically average between 6 - 8 mm while those of the *Wolffia* species are about 2 mm or less in diameter (Skillicorn, Spira, and Journey, 1993; Cheng and Stomp, 2009). In the winter months, the duckweed survive the low temperatures by forming a starchy survival frond known as a turion, which sinks to the bottom of the pond and remains dormant until spring (Skillicorn,

Spira, and Journey, 1993; Zirschky and Reed, 1988). The increase in temperatures in spring triggers their return to normal growth.

Growth conditions

Plant growth and reproduction is mainly affected by the availability of macronutrients such as nitrogen, phosphorus and potassium in addition to micronutrients, temperature, light, wave action and plant density (Culley et al., 1981; Lyerly, 2004; FAO, 1999). Duckweed is reported to be tolerant to a wide range of pH from 3 - 10 with an optimum range of 5 – 7 (Culley et al., 1981; FAO, 1999). The plants can also grow in a wide range of temperatures from 6 – 33°C with an optimum temperature range of 18 – 30°C (Culley et al., 1981; FAO, 1999).

Zirschky and Reed (1988) noted that duckweed growth can be limited by very high metal concentrations, presence of PCBs and ethylene as well as filamentous algae or fungus. Duckweed growth is also known to be highly sensitive to wind and wave action, as the wind blows the duckweed to the sides of the ponds where it piles up and subsequently dies (Iqbal, 1999). The effect of wind on duckweed systems not only affects the growth of the plants but also harvesting of the plant biomass. Wind effects on harvesting were clearly discussed by Smith (2003) in his research on harvesting duckweed by skimming. Over all, Culley et al. (1981) reported duckweed biomass doubling in 2 – 4 days under optimum growth condition.

Duckweed composition

Duckweed is composed of water, mineral elements, and organic matter. Fresh duckweed fronds have been reported to contain 87 to 97% water depending on the

species (Cross, 2006). Chemical analyses carried out on duckweed by Culley and Epps (1973) showed varying composition of crude protein, ash, fiber, water content, fat and mineral content depending on the harvest location, water source and species analyzed. The nutritional value of duckweed increased with plants grown in nutrient rich waters (Table 1) while mineral accumulation in the plant tissues depended on the aquatic habitat (Culley et al., 1981).

Studies have shown duckweed to assimilate phosphorus in the orthophosphate form (Culley et al., 1981). P removal efficiencies by duckweed systems were reported to range from 14 – 99% (Körner, Vermaat, and Veenstra, 2003). The plants' ability to uptake P depends on the growth rate, harvesting frequency and the available ortho-P (Iqbal, 1999). Gürtekin and Şekerdağ (2008) investigated the phosphate removal efficiency of *Lemna minor* in a secondary clarifier tank of a conventional biological treatment plant. The P removal efficiency directly attributed to the presence of duckweed in the settling tank was 45%. A similar study by Öbek and Hasar (2002) focused on the impact of harvesting duckweed on phosphate removal from secondary effluents. They realized 50% P uptake with no harvesting, 85.3% P uptake with a 5-day harvesting schedule and up to 96.7% P uptake with a 2-day schedule.

There is a growing concern of introducing organic pollutants, personal care products and pharmaceuticals into the food chain through duckweed based wastewater treatment systems (Reinhold, 2007). Shi et al. (2010) investigated the removal of EDC (estrone, 17 α-ethinylestradiol, and 17 ß-estradiol) in wastewater using duckweed and algae based systems. They realized removal efficiencies comparable to those of conventional activated sludge systems. It was also noticed that duckweed systems had a

higher efficiency at removing estrogens compared to algae systems. However the main removal mechanism was attributed to sorption and subsequent degradation by microorganisms. *L. minor* uptake of organic pollutants (fluorinated phenols) was reported to be rapid with a pseudo first order uptake rate of 0.2 – 0.84 d^{-1} in a study carried out by Reinhold (2007). He noted that the uptake rates did not correlate with commonly used pollutant properties like pKa, K_{ow}, and Hammett's constants. However, they were pollutant specific and appeared to depend on factors affecting the rates of plant metabolism (enzymatic processes) of organic pollutants.

Starch content in duckweed plants is highly variable. Cheng and Stomp (2009) reported starch values ranging from 3 – 75% of the dry weight. This variability was attributed to the different duckweed species and strains. They, however, reported the possibility of accumulating starch in the plant biomass to at least 25% of its dry weight. Accumulation of starch in the plant biomass is possible during periods of dormancy which can be achieved by varying growth conditions like pH, nutrient concentration, and temperature (Cheng and Stomp, 2009; Cui et al., 2010). McCombs and Ralph (1972) reported three times as much starch in non-growing duckweed plants left in the dark after 6 days compared to photosynthesizing plants in the same medium. This observation was also supported by Cheng and Stomp (2009) who realized 45.8% (dry basis) starch content in *S. polyrrhiza* species by transferring the plants from a nutrient rich solution (anaerobically treated swine waste) to a solution made up of tap water for 5 days. Cui et al. (2010) observed increased starch accumulation in *S. polyrrhiza* at a lower temperature of 5 $^{\circ}$C compared to 15 $^{\circ}$C and 25 $^{\circ}$C. This trend was further enhanced by a combination of low temperature with nutrient starvation.

Table 1. Typical chemical composition of duckweed cultured on nutrient-poor and nutrient-rich waters

	lagoon nutrient condition [a]	percent of dry wet									
		NFE[b]	crude protein	TKN[c]	Fat	Fiber	Ash	P	K	Ca	Mg
Spirodela punctata	Low		10.6	1.7	2.3	11.3	14.1	0.61	2.0	0.98	0.98
S.polyrrhiza	Low		13.1	2.1	2.5	16.1	13.3	0.56	2.4	1.21	0.76
Lemna gibba	Low		9.4	1.5	1.8	17	16.8	0.72	3.1	1.38	0.81
Spirodela punctata	High	33.2	36.8	5.9	4.8	9.7	15.2	1.50	2.8	1.75	0.84
S.polyrrhiza	High	31.8	39.7	6.4	5.3	9.3	12.8	2.10	3.4	1.28	0.92
Lemna gibba	High	31.1	36.3	5.8	6.3	10.1	15.5	2.60	4.4	1.81	0.88

[a]Low nutrient Lagoon contained less than 5 mg/L TKN. High nutrient lagoon contained greater than 30 mg/L TKN. Selected mean values from Culley et al. (1981)

[b]NFE (Nitrogen Free Extract, an estimate of carbohydrates)

[c]TKN (Total Kjeldahl Nitrogen)

Harvesting

Duckweed grows as a mat on the water surface making it very easy to harvest. A floating plastic grid system is recommended to prevent the plants from shifting from one side of a pond to another in case of windy conditions and to also prevent under or over harvesting of certain portions of the pond (Iqbal, 1999; Smith, 2003). Equipment used during harvesting range from a simple scoop or net, to specialized mechanical tools/harvesters made by the Lemna Corporation (Iqbal, 1999). Regular harvesting of the duckweed biomass is recommended in order to encourage plant growth and removal of dead or decaying plant material (Reed, Crites, and Middlebrooks, 1995). However, the quantity and frequency of the harvests should be driven by the nutrient removal requirements of the system and the need to maintain an optimum plant density on the

lagoons (Smith, 2003; Lyerly, 2004). The maximum duckweed productivity can only be achieved if the optimum standing crop density is determined and maintained during each harvest (Smith, 2003). Since the optimum standing crop density is site specific, it can only be determined through practical experience (Skillicorn, Spira, and Journey, 1993). The standing crop density can be determined using a calibrated fine mesh screen of 0.25 m^2 to gently lift a section of growing duckweed mat in the lagoon, with excess water drained and the collected biomass weighed with the screen. The standing crop density on square meter basis will therefore be determined as 4 times the weight recorded.

Re-use options

Understanding the plant's composition is pivotal in determining the biomass reuse options. There is a growing need to approach wastewater treatment and management processes in a holistic manner that addresses the need to preserve the environment and at the same time produce valuable byproducts that can sustain the system in place and the community. Duckweed based systems are no exception to this trend, and the biomass generated can be utilized in a number of ways as mentioned in the ensuing discussion. However, emphasis is not placed on the commercialization of the byproducts obtained but on increasing the attractiveness of the system as a wastewater treatment option. Therefore priority is given to meeting water quality requirements of the system, with beneficial byproduct generation as an added benefit.

Animal feed option. Duckweed has generated a lot of interest as a food source for fowl, ruminants, fish and humans around the world especially in developing countries (Iqbal, 1999). This has been attributed to its low fiber and high protein content, high protein quality and its protein yield per growing area (Cheng and Stomp, 2009). Typical

values reported in the literature on a dry weight basis show 15 - 25 % protein and 15 - 30 % fiber for duckweed grown on nutrient poor waters and 5 – 15% fiber and 15 - 45% protein for duckweed grown under ideal conditions depending on the species involved (Culley et al., 1981). This protein content is comparable to soybeans that range between 33 to 49% (Cheng and Stomp, 2009).

Human consumption of duckweed is rare although studies have shown the use of *Woffia arrhiza* in human diets in some parts of Burma, Laos and northern Thailand (Culley et al., 1981; Iqbal, 1999). On the other hand, duckweed use in animal diets as a sole or supplementary feed is widely documented. Feeding trials carried out on poultry, sheep, silver Perch, and Abalone by the RIRDC (1998) showed improved layer performance and egg quality in ducks and chickens, and no negative side effects observed in sheep. In brief they concluded that the animals willingly consumed the duckweed and it was beneficial to their growth. The most common application of duckweed as feed is found in fish farming. Skillicorn, Spira, and Journey (1993) document the successful cultivation and use of duckweed as a feed for Carp and Tilapia in Mirzapur, Bangladesh on a commercial basis. However, duckweed grown on wastewaters poses a concern to many due to the fear of pathogen transmission, heavy metal concentrations and toxins build up. In addition, the high water content limits the application of these systems to areas near farms for fear of increased handling, transport, and drying costs.

Most animals can be fed fresh duckweed but for some, like poultry, it is preferred that the plant biomass to be dried (Iqbal, 1999). RIRDC (1998) reviewed some technologies used to dry duckweed biomass such as; conventional drying ovens, solar power drying chambers, hydraulic presses and microwave technology. Pros and cons of

each technology were evaluated and it was concluded that using a solar powered drying chamber was the most economic drying method. Although solar drying was recommended by Culley and Epps (1973), it was noted that this method was highly dependent on the presiding weather conditions. It would therefore be more advantageous if gas was used to supplement solar heating in instances where duckweed is grown next to wastewater treatment plants with biodigesters (RIRDC, 1998). The length of the duckweed roots also seemed to affect the drying process; plants with long roots tended to mat up and dry slowly (Culley and Epps, 1973).

Fermentation for ethanol production**.** The fermentation process can be utilized to convert duckweed biomass into ethanol. The feasibility of this process mainly depends on the amount of starch present in the plant biomass. Starch is a polymer of glucose consisting of two structural components known as amylose and amylopectin: amylose is a linear polymer whose glucose residues are connected by α-1,4 linkages while amylopectin is a larger branched polymer consisting of both α-1,4 and α-1,6 linkages (Drapcho, Nghiem, and Walker, 2008) Starch can therefore be hydrolyzed to fermentable monomeric glucose sugars by the use of enzymes (Lin and Tanaka, 2006; Cheng and Stomp, 2009).

Ethanol production from starch involves a number of processes namely; gelatinization, hydrolysis and liquefaction of starch, and fermentation of the resulting dextrose (Lin and Tanaka, 2006). Gelatinization involves heating of the starch granules thereby weakening the hydrogen bonds and making the starch more water soluble. This process produces a highly viscous, amorphous gel that is more accessible to hydrolytic enzymes. The enzymes then hydrolyze and liquefy the starch present, thus reducing the

viscosity of the solution in addition to producing lower molecular size substrates such as glucose and maltose (Equation 1).

$$\text{Starch } (C_6H_{10}O_5)_n + \text{ n } (H_2O) \xrightarrow{\text{hydrolytic enzymes}} \text{n } (C_6H_{12}O_6) \quad (1)$$

Alpha-amylase hydrolyzes the 1,4-glucosidic bonds at random points in the starch molecule. The smaller sized sugars are readily degraded by microorganisms such as yeast (*Saccharomyces cerevisiae*) in the absence of oxygen in a process known as glycolysis (Embden-Meyerhof pathway, EMP), to produce energy in the form of ATP and ethanol as a byproduct (Equation 2) (Drapcho, Nghiem, and Walker, 2008).

$$\text{Glucose } (C_6H_{12}O_6) \xrightarrow{\text{glycolytic enzymes}} \text{Ethyl alcohol } (C_2H_5OH) + CO_2 + \text{ATP} \quad (2)$$

The theoretical yield of ethanol can be calculated using Equations 1 and 2. Even under ideal conditions, only 90-95% of the theoretical yield may be realized because not all the glucose consumed is converted to ethanol, as part of it is utilized for cell synthesis and production of other by products (Drapcho, Nghiem, and Walker, 2008). Fermentation is affected by factors such as: temperature, pH, ethanol concentration and substrate. The optimum pH required is 3.5 – 4 at a temperature of about 30 – 40 $^{\circ}$C.

Anaerobic digestion. Anaerobic digestion involves the microbiological breakdown of organic matter to methane and carbon dioxide in the absence of oxygen. This process occurs in four main stages namely: hydrolysis, acidogenesis/fermentation, acetogenesis and methanogenesis thereby requiring various groups of microbes responsible for each of the stages (Drapcho, Nghiem, and Walker, 2008). Acid forming bacteria convert the organics in the sludge to organic acids thereby decreasing the pH and carbonate alkalinity and increasing the volatile acid concentration. This trend is however

reversed by the methane forming bacteria which convert the organic acids to CO_2 and methane.

The microbial populations involved in these stages can only establish themselves if proper seeding, control of organic acid build up, and optimum pH are maintained during start-up and operation of the digestion system (El Fadel and Maroun, 2007). The start-up period has been reported to take 3 weeks to a year (El Fadel and Maroun, 2007). Digester stability and health is determined by monitoring pH, VFAs, and alkalinity. pH is easily measured and thus can be monitored on a daily basis. The measured pH is both a result of the level of alkalinity and the rate of acid formation in the reactor. An average pH range of 6.7 – 7.4 is desired for a healthy digester (Zhao and Viraraghavan, 2004; Drapcho, Nghiem, and Walker, 2008). A pH drop below 6.7 upsets the microbial population balance resulting in VFA accumulation. The pH drop may be as a result of sudden changes in loading rates, temperature or feed composition and can be corrected by introducing a base, usually $CaCO_3$ or NaOH solution.

Anaerobic digestion processes have been reported to operate over a wide range of VFA concentrations (i.e., from 100 mg/L to over 5,000 mg/L) provided the proper pH range is maintained (Droste, 1997). Alkalinity is mainly from the destruction of organics containing nitrogen forming ammonia that reacts with the carbon dioxide to form ammonium bicarbonate as illustrated by Eq. 3 (Metcalf and Eddy, 2003).

$$NH_3 + H_2O + CO_2 \xrightarrow{\text{yields}} NH_4^+ + HCO_3^- \quad (3)$$

The ratio of volatile acids to alkalinity should be between 0.1 and 0.2 (Sung and Santha, 2003; Zhao and Viraraghavan, 2004). A ratio greater than 0.8 indicates a process failure while a ratio between 0.3 – 0.4 indicates an upset in the process requiring corrective measures (Zhao and Viraraghavan, 2004). Ammonium can be tolerated up to 1500 - 3000 mg/L as NH_4-N at a pH above 7.4; however, free ammonia above 80 mg/L can inhibit the anaerobic digestion process (Metcalf and Eddy, 2003). Furthermore, the rate of organic matter conversion can be determined by monitoring total and volatile solids concentrations in anaerobic digesters.

Literature on the use of duckweed as a sole feedstock for anaerobic digestion is not readily available. Most studies utilize duckweed to enhance the anaerobic process in a co-digestion system. Clark and Hillman (1996) investigated the impact of adding iron rich duckweed as a supplement to chicken manure in batch and semi-continuous lab scale anaerobic digesters. They observed improved nutritional balance resulting in an increased gas production rate of about 44%.

Based on literature review of the available solid management options, anaerobic digestion was considered a more attractive solids management option for duckweed compared to land filling and incineration. This was because of the high energy costs involved in the incineration process, and the potential increase in leachate produced in landfills as a result of high moisture content in the duckweed biomass (UN, 2003). Advantages of anaerobic digestion include: rapid stabilization of organic matter, reduction of waste volume leading to less land requirement for solids disposal, production of energy, and digester waste that can be used as a soil conditioner (Chynoweth, 1987; El Fadel and Maroun, 2007). The research carried out in this study

was done using mesophilic digestion (35 – 40 °C) at a retention time of 20 days rather than thermophilic digestion (50 – 55 °C) because mesophilic systems are more robust, and are less expensive and energy intensive than their thermophilic counterparts.

Waste Disposal

Removal of nutrients and contaminants of concern from a lagoon using a duckweed-based system involves harvesting of the biomass. This biomass can be processed and subsequently managed using one of the above mentioned options. The harvested biomass is not considered biosolids, and as such the biosolids regulations do not apply (Mark Schmitz, Biosolids coordinator DEQ Utah, personal communication, January, 06, 2011). It is still important to keep track of the fate of pollutants throughout the system and the biomass disposal process. For instance, after anaerobic digestion, P concentrated in the waste effluent stream can be precipitated out as struvite and used as a fertilizer. Additionally, the residual digester solids can be used as a nutrient rich soil conditioner. On the other hand, if the biomass is used as an animal feed, the P is consumed by the animals and eventually gets into the food chain or is disposed of from their bodies through excreta. Regardless of the option chosen, it is important to monitor the pollutants of concern within the system and where possible close the cycle so that the system is more sustainable (Figure 2).

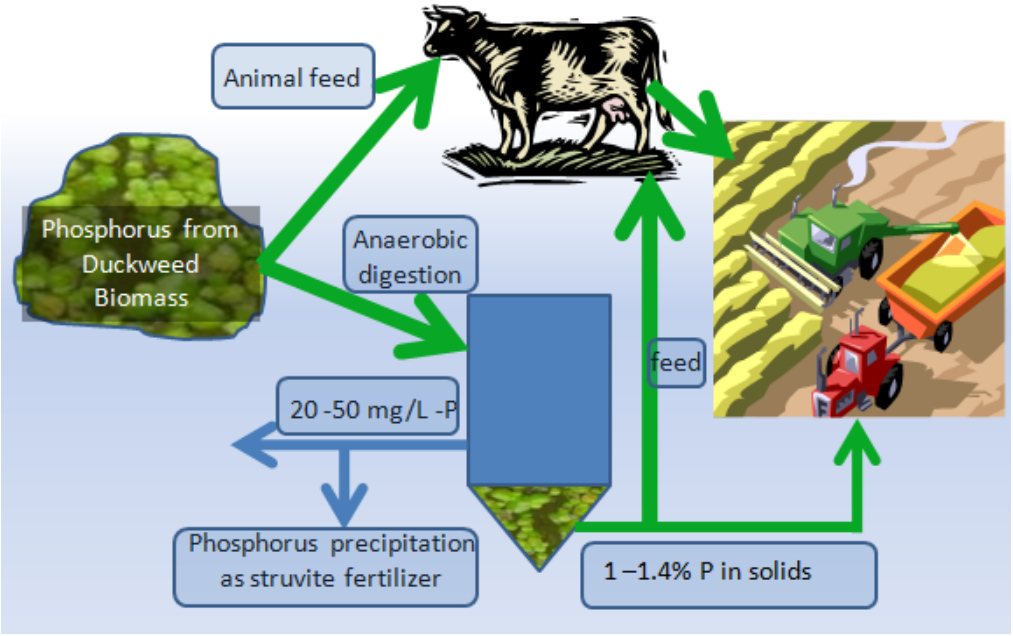

Figure 2. The fate of P in a duckweed-based system.

MATERIALS AND METHODS

This chapter describes the methods and activities that were undertaken to achieve the objectives of the study. Details of these are the subject of the subsequent sections. Manually harvested duckweed from the Wellsville Lagoons was brought to the UWRL in a 5-gallon container for anaerobic digestion, fermentation and animal feed analysis. Harvesting was done at least once a week using a net during the summer and early fall months. A fraction of the harvested biomass was air dried to 5 -10% moisture content, ground and then stored in ziplock bags until use. The other fraction was placed in a container with wastewater from the lagoon and used as a source of fresh duckweed for the digesters and fermenters.

Animal Feed Option

Duckweed composition

Chemical analysis of the duckweed biomass was carried out by the Utah State University Analytical Lab, (Logan UT) and Huffman Laboratories, Inc. (Colorado) to determine its composition. In addition, an in vitro fermentation procedure was carried out by the Utah State University Skaggs Nutritional Laboratory (Logan UT) to determine the digestibility of the duckweed biomass. For the in vitro fermentation procedure, the harvested duckweed was freeze dried to prevent any loss of nutrients using a Labconco Free zone Plus Freeze Dry system to a temperature of about – 82 $^{\circ}$C. The Daisy II in vitro fermentation system (ANKOM Corp, Macedon NY) was used to determine duckweed digestibility (Figure 3). The procedure described by Colombatto et al. (2003) was used with the exclusion of the four enzyme products.

Figure 3. Daisy ^{II} incubator (Skaggs Nutrition Lab) permission granted by Dr. Jong-Su Eun.

The incubator was maintained at 39 $^{\circ}$C ± 0.5 $^{\circ}$C and the incubation was carried out for 96 h. The following treatments were considered in this study: Duckweed, Alfalfa, Corn silage, and combinations of duckweed-alfalfa, duckweed-corn silage and duckweed-alfalfa-corn silage.

Anaerobic Digestion

Reactor configuration

Two completely mixed, batch fed reactor systems were utilized. Mixing loosened up the gas bubbles and allowed homogenous mixing of the contents. Each reactor system was comprised of a 2-L glass bottle connected to a gas collection system by plastic Tygon tubing (Figure 4). The lid was connected to an off gas line and a feed line, while

the bottom part of the glass bottle was used as a sampling port. The gas collection system included two 1-L water – displacement graduated plastic containers connected by plastic Tygon tubing. The containers were filled with acidified gas collection solution mixed with methyl red indicator for ease of reading. The gas collection liquid was prepared by dissolving 4 g/L sodium chloride in 5% sulfuric acid solution to prevent biological growth and minimize CO_2 solubility in the liquid. The reactors were placed on magnetic stirrers in an incubator at a constant temperature of 35 °C.

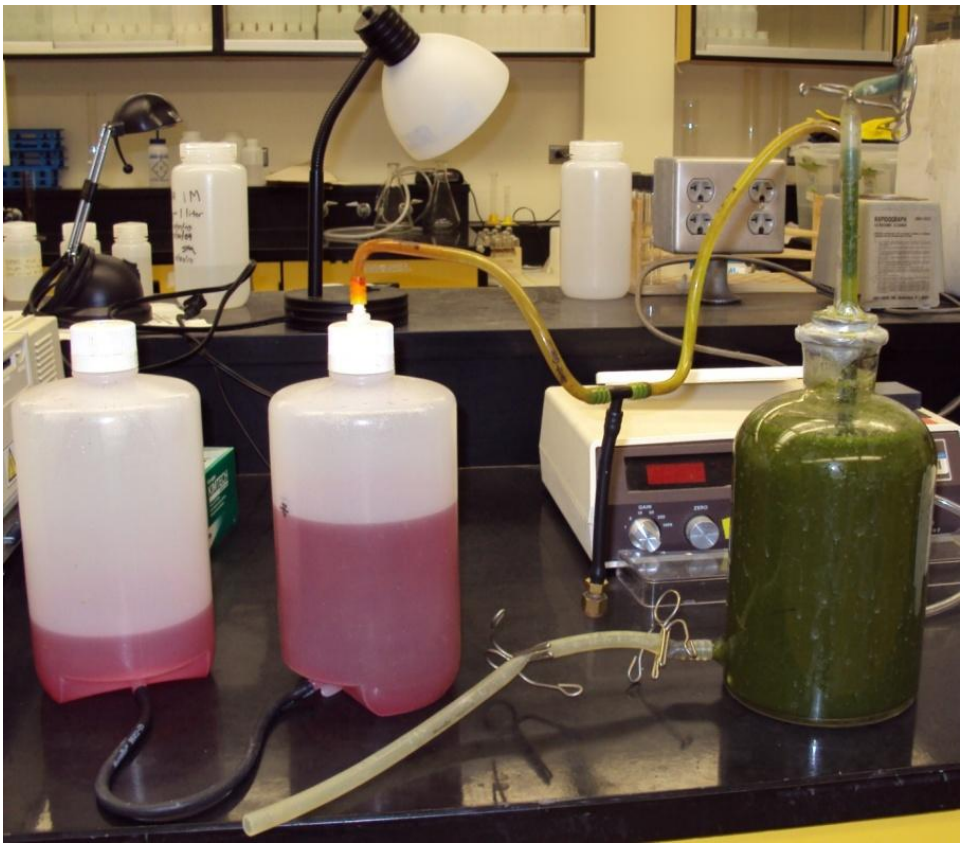

Figure 4. Reactor configuration for anaerobic digestion.

Procedure

The startup material in the reactors included; 60 g (dry wt) of duckweed biomass, 500 mL of sewage sludge as the inoculum and 1 L of tap water. Fresh and dry ground duckweed biomass was fed to each reactor, respectively. The initial pH of the mixture was measured and recorded. A 0.0125M NaOH solution was used to keep the pH within the desired range (6.5 -7.7) in the subsequent days until pH stability was attained. One gram (dry wt) of ground biomass was manually fed into the digester every other day using a syringe until steady state conditions were obtained. The feeding load was then gradually increased by 0.5 g increments until a maximum loading value was obtained. In order to determine the maximum loading value, the digester pH was monitored to identify when the digester began to fail (pH below 6.7). The amount of feed that caused the digester to fail was considered the maximum loading value. Feeding and sampling were done simultaneous such that the volume of effluent withdrawn was the same as the volume fed. The reactors were operated at a hydraulic retention time of 20 days. The biomass was ground to ease feeding and reduce the possibility of oxygen entering the digesters during the feeding process. Each feed batch was measured for moisture content, and volatile and total solids. The amount of gas produced was equivalent to the volume of liquid displaced by the gas. The reported gas volume was corrected to Standard Temperature and Pressure (STP) conditions.

Gas samples were collected using a syringe from the gas sampling port and analyzed. Analysis of gas composition was done using gas chromatography (GC) by direct injection into an Alltech CTR-1 column with a thermal conductivity detector. The

GC was calibrated against a $CH_4/CO_2/O_2$ standard of known composition. Gas samples were taken every other day.

The digester effluent was analyzed for pH using a calibrated pH meter. TS and VS were determined according to Method 2540 B and Method 2540 E, respectively, as described in the Standards Methods (APHA/AWWA/WEF, 2005) every other day. Digester effluent was analyzed for alkalinity once a month according to Method 2320B (APHA/AWWA/WEF, 2005). The Dionex DX-500 ion chromatography system with a Peak Net workstation was used to separate and measure the cations and anions of interest. The system for NH_3-N analysis was comprised of an IC 25 Ion Chromatograph, AD 25 absorbance detector, AS40 automated sampler, LC20 chromatography enclosure, a PC 10 pneumatic controller and a cation self re-generating suppressor (CSRS 300 x 4mm, P/N 064556). A Reagent Free ^{TM} Ion Chromatography (RFIC) system equipped with Ion Pac^{R} CS12A (4 x 250 mm) analytical column P/N 064556, and a CG12A (4 x 50 mm) Guard was used in the analysis. The eluent was 0.03N H_2SO_4 at a flow rate of 1.00 mL/min. A 50 µL sample volume was used. The samples were analyzed following the Dionex Application Note 141 for inorganic cations and ammonium. Volatile fatty acids were analyzed using an ion chromatography Dionex Application Note 123 for inorganic anions and VFAs. The following VFAs were included in the procedure; lactate, acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, succinate, fumurate, citrate, and isocitrate. The system was comprised of a GP 40 gradient pump, CD 20 conductivity detector, AS 40 automated sampler, a RFIC ^{TM} Ion Pac ATC-HC (9 x 75 mm) trap column and an ASRS 300 x 4 mm P/N 064554 anion self re-generating suppressor. The eluents used included; 100mM NaOH and 5mM NaOH at a flow rate of 1.5 mL/min. A

50 µL sample volume was used. Phosphorus in the duckweed biomass samples was determined from USU analytical lab results and a modified lab procedure obtained by combining dry ash and wet ash tissue digestion procedures with aqua regia soln and ascorbic acid Method 4500-P from Standard Methods (APHA/AWWA/WEF, 2005). Details of the procedure are given in Appendix D. Laboratory analysis of the samples was carried out at the Utah Water Research Lab (UWRL).

Fermentation for Ethanol Production

Determination of starch content

Part of the harvested biomass was transferred to another container containing nutrient free water and left to sit for 5 - 6 days. This was done to allow for conversion of the stored nutrients into starch by the duckweed. Starch content at Day One and Day Six were measured in order to determine the percentage increase in starch during this "ripening" period. The duckweed biomass was then ground and processed for the fermentation process in the LiFlus GX bioreactor. Starch measurements were done according to the AOAC Method 996.11 and AACC Method 76.13 using the total starch assay procedure (Amylogucosidase/α-Amylase) from Megazyme International, Wicklow (Ireland).

Reactor configuration

The set up consisted of an autoclavable 3 L double vessel LiFlus GX-Bioreactor (Serial no. GX-0612F – 46, Bio Tron Inc, Korea) connected to a water bath system (Figure 5). The water bath temperature was set and maintained at 35 $^{\circ}$C throughout the fermentation period. A DC motor was mounted on top of the head plate to control the

agitation speed. The head plate consisted of several ports that were sealed off except for the sampling port, a port for the pH meter and thermometer, and an inlet for a NaOH solution. The controller body was comprised of an LCD monitor that displayed all the measured values and controlled parameters, and four peristaltic pumps. Only one pump was utilized to supply NaOH to the fermenter when the pH levels dropped below the desired setting. The pH meter was calibrated and all controls set before the start of the fermentation process.

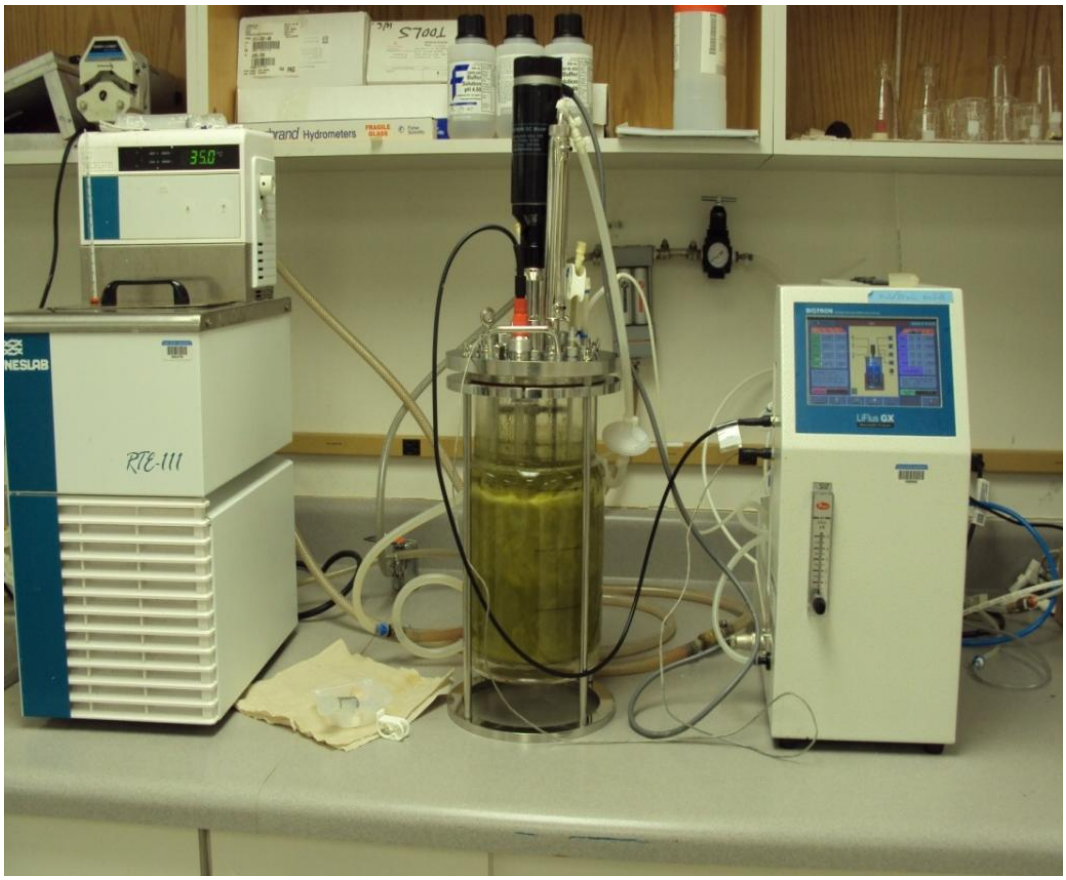

Figure 5. Bioreactor for fermentation.

Procedure

The 6-day biomass was harvested and excess water was gravity drained. Blending of the fresh biomass was done at a high speed for 3 – 5 minutes to ensure uniformity and complete mixing of the slurry. The biomass mixture was transferred to a 3 L beaker and placed on a hot plate to boil at a temperature of 95 – 100 for about 30 minutes. The slurry was continuously stirred during the heating process. Heating gelatinizes the starch in the biomass and also sterilizes the resulting solution. The solution was then cooled to 70 – 80 $^{\circ}$C, and maintained at this temperature for 30 minutes. α-Amylase enzyme obtained from a local brewer's shop was added to the mixture while still stirring. Addition of α-Amylase helps in liquefaction of the starch gel and hydrolysis of starch to dextrins. Complete conversion of starch to sugar was tested by use of an iodine solution. A blue-black color change signified the presence of starch and a reddish color change was an indication of the absence of starch.

At the end of the 30-minute cooling period, the duckweed slurry was transferred to the 3 L bioreactor at a temperature of 30 - 38 $^{\circ}$C. One gram of brewer's yeast (Pasteur and Premier Cuvee Wine Yeast brand) obtained from a local brewers' shop was added, and the pH of the slurry was adjusted to 3.5 – 4.5. The bioreactor was closed off and a run was started for 48 hours. Samples were obtained at t = 0, 6, 12, 18, 24, 36, and 48 hrs for ethanol content analysis. pH was automatically adjusted during the run. Sample preservation was by freeze drying.

Ethanol measurements

Ethanol concentrations were measured using headspace gas chromatography with mass spectrometer detection. A 10-mL sample was placed in a 22-mL headspace vial,

and run through a GC/MS system via a Tekmar HT7000 headspace analyzer. The conditions for the HT7000 were set to 10 min with agitation at 50 °C, 40 psi pressurization, and loading of a 1-mL sample loop. The GC oven conditions were 35 °C for 1 minute, ramp at 20 °C/min to 170 °C, no hold, and a final ramp at 30 °C/min to 220 °C with a hold for 5 minutes. A 30 m x 1.7 µm film x 0.25 mm ID DB-624 capillary column was used. The mass spectrometer was operated in +EI mode, in scan mode over a range of 35-60 amu at approximately 5 scans/sec.

Data Reduction Methods

Data collected during the course of the research was analyzed and processed in order to meet the objectives of the study. The following procedures were used to analyze data and the subsequent results used in the discussion of results.

Duckweed molecular formula determination

Chemical and elemental composition analysis of the duckweed was carried out by the Utah State University Analytical lab, Logan Utah and Huffman Lab, Colorado respectively (Appendix A).

In order to determine the molecular formula of the duckweed, the percent weight composition of each element obtained from the elemental composition analysis was used in the calculation. Average percent elemental composition on a wt% basis was found to be: 36.82% C, 4.78% H, 3.97% N, 28.81% O, 1.03 % P, 0.81% S, and 24.81 % ash (Table 2). Ignoring sulfur and ash components the resulting formula for ash free duckweed biomass can be calculated as

	C	H	O	N	P
Moles	$\frac{36.82}{12}$	$\frac{4.78}{1}$	$\frac{28.81}{16}$	$\frac{3.97}{14}$	$\frac{1.03}{31}$
Whole No. ratio	$\frac{3.07}{0.03}$	$\frac{4.78}{0.03}$	$\frac{1.8}{0.03}$	$\frac{0.28}{0.03}$	$\frac{0.03}{0.03}$
	102	159	60	9	1

Therefore the resulting duckweed formula is $C_{102}H_{159}N_9O_{60}P$ on a VS basis.

Table 2. Summary of duckweed chemical and elemental composition results

	Skaggs LAB	USU analytical LAB (S.lagoon 1)	USU analytical LAB (N.lagoon 2)	USU analytical LAB (S.lagoon 3)	USU analytical LAB (S.lagoon4)
organic matter	78.50	-	-	-	-
crude protein	23.00	27.80	38.40	21.40	21.80
Neutral Detergent Fiber (NDF)	30.20	34.60	26.10	30.70	28.40
Acid Detergent Fiber (ADF)	13.70	24.30	16.90	20.00	20.50
Acid Detergent Lignin (ADL)	4.81	-	-	-	-
Cellulose (ADF- ADL)	8.89	-	-	-	-
Hemicellulose (NDF-ADF)	16.50	10.30	9.20	10.70	7.90
*Carbon		40.32	36.77	35.31	34.89
*Hydrogen		5.3	4.78	4.52	4.53
*Nitrogen		5.15	4.15	3.22	3.37
*Oxygen		29.45	27.06	29.36	29.36
*Ash		19.04	26.48	26.69	27.01
Phosphorus		0.98	0.83	1.23	1.10
*Huffman Lab, all percentage values based on dry weight					

Theoretical gas production from
anaerobic digestion of duckweed biomass

The theoretical gas production value was determined using a generalized formula representing the anaerobic digestion process of organic constituents of solid waste.

$$C_aH_bO_cN_d + \left(\frac{4a-b-2c+3d}{4}\right) H_2O \xrightarrow{\text{yields}} \left(\frac{4a+b-2c-3d}{8}\right) CH_4 + \left(\frac{4a-b+2c+3d}{8}\right) CO_2 + dNH_3 \quad (4)$$

Substituting the organic components with the duckweed biomass formula obtained above;

$$C_{102}H_{159}O_{60}N_9 + \left(\frac{4*102-159-2*60+3*9}{4}\right) H_2O \xrightarrow{\text{yields}} \left(\frac{4*102+159-2*60-3*9}{8}\right) CH_4 + \left(\frac{4*102-159+2*60+3*9}{8}\right) CO_2 + 9NH_3$$

Using stoichiometry, the resulting balanced equation can be solved to obtain the expected gas production values under ideal conditions.

$$C_{102}H_{159}O_{60}N_9 + 39\ H_2O \xrightarrow{\text{yields}} 52.5\ CH_4 + 49.5\ CO_2 + 9NH_3 \quad (5)$$

For one mole of gas at STP, (1 mole of gas occupies 22.4 L at 0 $^{\circ}$C and 1 atm)

$$\text{Gas production} = \frac{22.4 * 52.5\ \text{L}\ CH_4}{2469\ \text{g duckweed}} = 0.476\ \text{L}\ CH_4/\text{g dry duckweed VS}\ (7.62 \frac{\text{ft}^3}{\text{lb VS}})$$

Laboratory gas production values were compared to the theoretical value obtained in order to determine the performance of anaerobic digestion process. Total gas production was estimated from the percentage of volatile solids reduction. Typical methane production values vary from 0.75 – 1.12 m^3/kg (12 to 18 ft^3/lb) of volatile solids destroyed (Metcalf and Eddy, 2003).

Theoretical COD of duckweed biomass

Duckweed biomass was expressed on a COD basis as follows;

$$C_{102}H_{159}O_{60}N_9 + 105\ O_2 \xrightarrow{\text{yields}} 66\ H_2O + 102\ CO_2 + 9\ NH_3 \quad (6)$$

$$\text{COD} = \frac{105 * (2 * 16)\text{g/mole}}{2469\ \text{g/mole}} = 1.36\ \frac{\text{g}\ O_2}{\text{g dry duckweed VS}}$$

The measured COD value of the duckweed was compared to the theoretical value obtained.

Theoretical ethanol production from fermentation of duckweed biomass

Determination of the theoretical ethanol production provided a means of comparing the measured laboratory values to the expected values under ideal conditions. Using Equations 1 and 2;

$$\text{Starch}\ (C_6H_{10}O_5)_n + \text{n}\ (H_2O) \xrightarrow{\text{hydrolytic enzymes}} \text{n}\ (C_6H_{12}O_6)$$

For 1 g of starch in duckweed biomass

$\text{Glucose amount} = \frac{180\text{n}}{162\ \text{n}}$ =1.1111 when n is large; therefore 1.111 is the factor used to theoretically determine glucose production by starch hydrolysis.

$$\text{Glucose}\ (C_6H_{12}O_6) \xrightarrow{\text{glycolytic enzymes}} 2\ (C_2H_5OH) + 2CO_2 + \text{ATP}$$

$$\text{Ethanol production} = \frac{2 * 46}{180} = 0.511\ \text{g ethanol per g starch in duckweed}$$

Sample calculations for ethanol yields are presented in Appendix B.

Determination of VS destruction through anaerobic digestion of duckweed biomass (VS mass balance)

Methane yield was determined as a ratio of volume of methane produced per mass of volatile solids destroyed. The measure of volatile solids destroyed throughout the process was determined by carrying out a mass balance on the volatile solids in the system using the anaerobic digestion results obtained.

$$\text{VS fed on day 1 } (\text{VSo})_{\text{in}} - \text{VS before feeding at day 2 } (\text{VS}_{\text{end}}) = \Delta\text{VS} \quad (7)$$

For any given day:

$$\text{VSo, mg} = \left(\text{Day 1 effluent VS before feeding } (\frac{\text{mg}}{\text{L}}) * 1.8\ \text{L}\right) + \left(\text{Day 1 feed VS added (mg)}\right)$$

$$\text{VS}_{\text{end}}, \text{mg} = \left(\text{Day 2 effluent VS before feeding } (\frac{\text{mg}}{\text{L}}) * 2\text{L}\right)$$

In order to maintain a 20 day HRT for the reactors, an effluent volume of 0.2 L was taken out of the reactor every other day. This was taken into account by using a volume of 1.8 L in the calculations shown above. Subsequently, the digester volume was always topped up to the 2L mark with feed.

Percent VS reduction was calculated as;

$$(\%\ \text{vs reduction}) = \frac{\text{VS}_{\text{feed}} - \text{VS}_{\text{sludge}}}{\text{VS}_{\text{feed}}} \text{ x } 100 \quad (8)$$

Sample calculations for %VS reduction for Reactor 2 are presented in Appendix B.

RESULTS AND DISCUSSION

Composition of Duckweed

Duckweed species analyzed were composed of: 94 - 96 % water (Figure 6), less than 10% starch (Table 3), 21- 38 % crude protein (Table 4), and 78.5 % organic matter (Table 5), and a number of minerals as shown in the detailed lab reports (Appendix A). Special emphasis was placed on crude protein, starch content and water content values because of the influence of these parameters on the biomass management alternatives considered. Raw results from the anaerobic digestion process are presented in the attached CD according to the format shown in Appendix E.

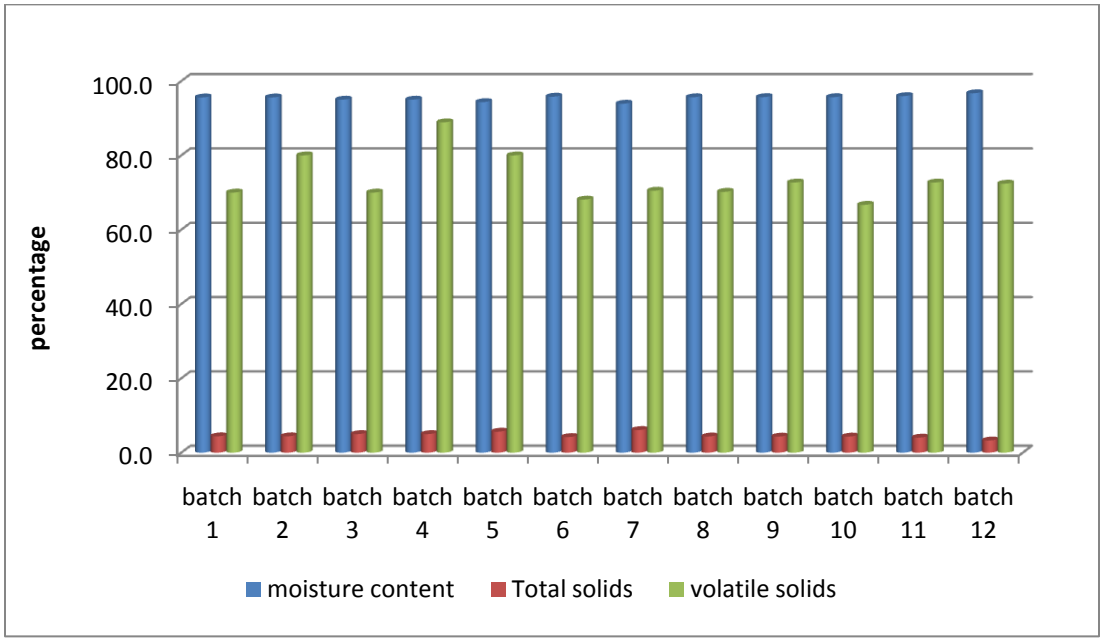

Figure 6. Duckweed moisture content and solids content results.

Table 3. Average starch content in lab grown fresh and oven dried duckweed biomass

Duckweed	day 1
Fresh	8.88 ± 4.61
Oven dried	2.59 ± 1.79

Table 4. Comparison of Duckweed composition results with previous study values in literature

Species	Water content % dry wt	crude protein % dry wt	Starch, % dry wt				
			Do nothing	Nutrient starvation	*at 25°C	*at 5°C	*at 15°C
Wellsville WWTP Mixed culture (L.minor and Woffia)	94 – 96	21 – 38	<10	6 - 38	N/A	N/A	N/A
Lemna gibba	93 - 95.4	25.2 - 36.3	N/A	N/A	N/A	N/A	N/A
S.punctata	94.8	28.7 - 36.8	N/A	N/A	N/A	N/A	N/A
S.polyrhiza	94.9	29.1 - 39.7	<10	23.9 - 45. 8	6.51 - 7.87	8.78 - 15	8.01 - 11.2
Typical range	86 – 97	15.0 – 45.0	3 - 75				

Starch values Cheng and Stomp (2009) and Cui et al. (2010)
* range inclusive of values for obtained at residence times of 0 - 6 days (Cui et al., 2010)
Crude protein and water content values Blakeney, Culley, and Rusoff (1980), Culley et al. (1981)

Table 5. Comparison of duckweed chemical composition to other common ruminant forages using in vitro fermentation results

Chemical composition (% of dry matter), Skaggs Nutritional Lab (USU)			
Item	Alfalfa hay	Corn silage	Duckweed
organic matter (OM)	89.3	94.7	78.5
crude protein (CP)	19.2	6.25	23.0
Neutral detergent fiber (NDF)	37.0	42.2	30.2
Acid detergent fiber (ADF)	27.4	22.7	13.7
Moisture content (MC)	75 – 83	60 – 70	92 – 94

The water and crude protein (CP) content in the duckweed species studied was compared to that obtained in previous studies by Blakeney, Culley, and Rusoff (1980) and Culley et al. (1981) on duckweed species from different growth conditions. The CP values and moisture content observed in this study was within the expected range and comparable to results reported in literature (Table 4). The starch content in the duckweed was highly variable with less than 10% starch observed in the plant biomass (Table 3). The low starch values observed were comparable to results reported by Cui et al. (2010) for duckweed grown at 25 $^{\circ}$C (Table 4). Although low starch values were realized, they were still within the range reported by Cheng and Stomp (2009) for starch in duckweed plants (Table 4). An attempt was made to accumulate starch in the duckweed by placing the plant biomass in nutrient deficient media. An average percent starch value of 19 ± 11.03% was obtained after nutrient starvation (Table 6). This value was lower than that observed by Cheng and Stomp (2009) and Cui et al. (2010) after nutrient starvation (Table 4).

Table 6. Starch measurement of 6d duckweed biomass grown on nutrient deficient media

14-May-10	6.31
18-Jun-10	24.40
23-Jun-10	38.49
26-Jun-10	28.95
23-Jul-10	18.32
31-Jul-10	11.61
13-Sep-10	8.04
20-Oct-10	16.41
Average	19 ± 11.03

Animal Feed

The nutritive value of feed for ruminants is determined from the concentration of its chemical components, the rate and extent of its digestion, and the animal's intake (Mertens, 2000; Getachew et al., 2004). Feed analysis reports (Appendix A) and results from the in vitro fermentation digestibility studies (Table A-1, A-2, and A-3) obtained in this study were utilized to determine the quality of duckweed as a ruminant feed.

Duckweed was found to contain 23% crude protein on a dry weight basis (Table 5). The CP values give an estimate of the total protein content of a feed based on the nitrogen present, i.e., CP = % nitrogen x 6.25 (6.25 accounts for an average of 16% nitrogen contained in proteins). Therefore CP is comprised of both the true protein (amino acids) and non protein nitrogen such as urea and ammonia nitrogen. Crude protein content in duckweed was found to be higher than that of alfalfa hay and corn silage in this study (Table 5). A high CP value is desired in animal nutrition because the value of a feed is directly proportional to its crude protein content (FAO, 1999).

Neutral and Acid Detergent Fiber values are useful in evaluating forage and formulating rations. The NDF value consists of three components of a plant cell wall namely; cellulose, hemicellose and lignin, while the ADF consists of cellulose and lignin. From the NDF value an estimate of the total fiber content in a feed can be determined. Low NDF values correlate with higher amounts of feed consumed by an animal. This is because it is the fiber part of the feed that limits digestion, requires chewing for particle size reduction and occupies space in the rumen (Grant, 1991; Mertens, 2000). On the other hand, ADF values give a measure of the least digestible portion of the feed, therefore low ADF values show increased digestibility of the feed. The NDF and ADF

values obtained for the duckweed were 30.2% and 13.7%, respectively (Table 5). The NDF and ADF values of duckweed were compared to those of alfalfa hay and corn silage and it was found that duckweed had the lowest values of them all (Table 5). This showed that duckweed is a great potential feed for ruminants. Similarly, using the Utah feed values, duckweed was categorized as a supreme feed, the same category as alfalfa hay and corn silage (Table C-1).

The relative feed value (RFV) ranks a feed based on its digestibility (ADF) and its intake potential (NDF). RFV is used in marketing and comparing of feeds; the higher the RFV the better the forage quality. In Utah, the desired RFV should be above 185 which represents 22-23% protein, 26–27% ADF and 33-36% NDF (UDAF, 2010), and as a guideline any value within ± 5 points of the target value is acceptable. The RFV calculated for duckweed was in the range of 230–241 (Appendix B).

The percent ADF, NDF and dry matter (DM) degradability of duckweed samples was obtained and compared to that of corn silage and alfalfa hay for incubation times of 6, 12, 36, 48 and 96 h (Table A-1). The higher the degradability value obtained, the better the feed. This is because feed with high degradability take less time in the rumen thereby increasing intake and amount of nutrient absorbed by the animal. Degradability generally increases with time of incubation (Table A-1). Duckweed dry matter degradability was lower than expected and significantly different from that of alfalfa and corn silage (Figure 7). At 2 – 12 h, the DM degradability of duckweed was higher than corn silage but lower than alfalfa hay, while at 24 – 96 h, it was lower than both corn silage and alfalfa hay. A study by Johnson (1998) also observed low DM degradability values from duckweed grown on municipal wastewater compared to that grown on animal waste. He

attributed this to the possibility of duckweed lacking the needed energy to digest the amount of dry matter it contains. Acceptable DM digestibility for feed used as protein supplements is 60% and above (Johnson, 1998).

The NDF digestibility gives a better estimate of the total digestible nutrients (TDN), net energy (NE) and feed intake potential (Juárez et al., 2004). NDF degradability values for duckweed at 6 – 12 h were significantly higher than those of corn silage and alfalfa hay (Figure 8). This may have been due to the low amounts of lignin observed in duckweed (Table A-3). Over all the DM, ADF and NDF degradability values observed for the duckweed was 60%, 40% and 50%, respectively (Table A-1). The overall duckweed digestibility was slightly lower than that of alfalfa hay and corn silage.

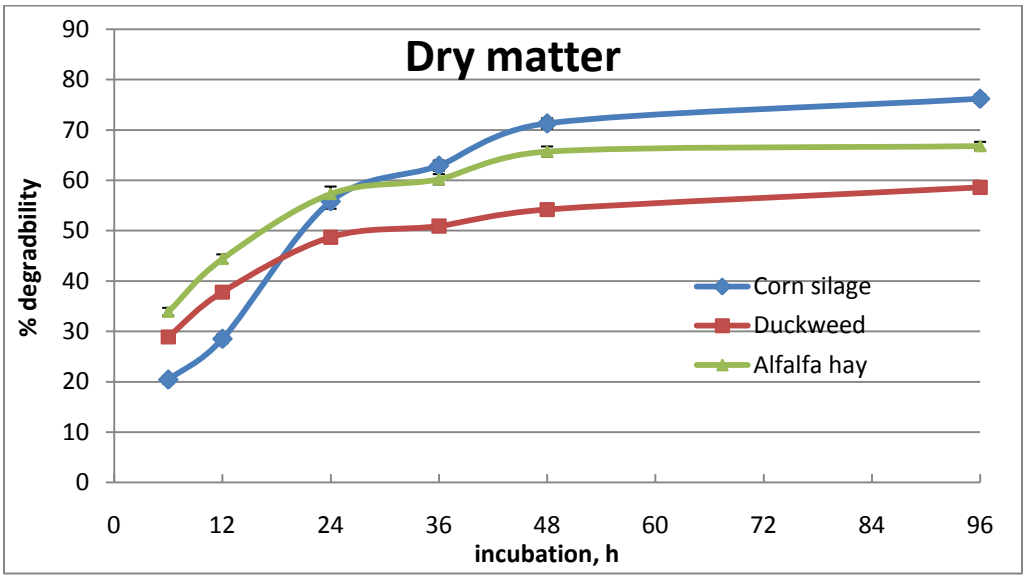

Figure 7. % DM degradability of duckweed compared to alfalfa and corn silage by in vitro fermentation.

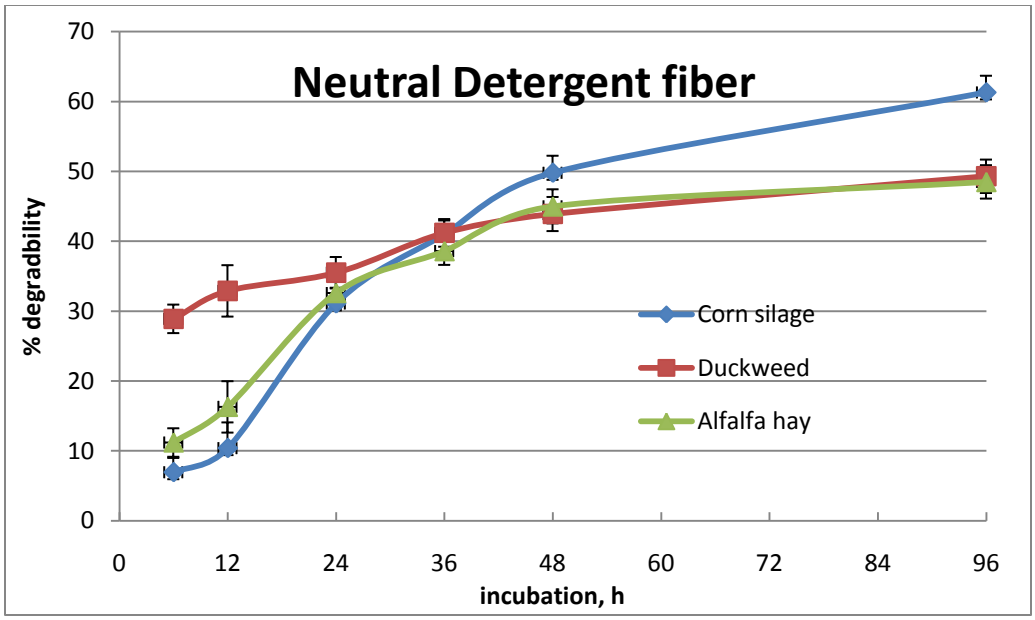

Figure 8. % NDF degradability of duckweed compared to alfalfa and corn silage by in vitro fermentation.

Another important factor to note if duckweed is to be utilized as a feed is its mineral content. Small amounts of minerals are useful in a feed but high concentrations may result in toxicity depending on the dosage applied. The mineral content in duckweed can enhance its use as a feed or potentially defeat this purpose. P, Zn, Cr, Co, Cu, and Ni levels observed did meet the recommended amounts required for beef cattle by NRC (1996) (Appendix C). Ca, Fe, K, and Mg levels in the duckweed exceeded the maximum tolerable limit for beef cattle (NRC, 1996). Mo levels were non detectable thus below the required amounts for beef cattle and therefore would need to be supplemented (NRC, 1996). It is possible that Al and Cd levels detected in the duckweed may be toxic to beef cattle (Table C-2). However, most of these concerns can be worked out with a nutritionist so that a balanced feed is produced that will not harm the animal's health.

Heavy metal accumulation in duckweed biomass has been reported in plants grown on industrial or mining waste (FAO, 1999). Municipal or animal waste sources

usually have low concentrations of metals and as such heavy metal accumulation by duckweed is usually not a problem (Ramjeet-Samad, 2010). In cases where this issue does arise, the duckweed biomass should be disposed off according to regulations and not fed to animals. It is therefore recommended that the source and composition of the wastewater be known before the animal feed option is implemented.

There is limited information on pathogen transmission from wastewater grown duckweed utilized as feed to ruminants and thus there is a need for further research in this area. Skillicorn, Spira, and Journey (1993) and Cross (2006) suggested providing a sufficient retention time in clean water to ensure that harvested biomass is free from water borne pathogens, thereby minimizing pathogen transmission to the animals. Pathogen reduction would be mainly by sedimentation, die off and dilution. They, however, did not specify the actual time required for this process.

Anaerobic Digestion

Start up and acclimatization period

Start up was first attempted using cow manure as an inoculum. The reactors were left to settle for a week with only gas production and pH levels being monitored. Within the week there was a drastic drop in pH and the reactors quickly turned sour. Efforts were made to increase the pH levels by addition of calcium carbonate to the slurry and no feeding for at least 2 weeks, with no significant improvement noticed. Use of sodium bicarbonate is recommended instead of calcium carbonate that easily precipitates out of solution with continuous use depending on the pH and alkalinity (Gerardi, 2003). A decision was then made to change the starter material after a number of failed attempts. It

was believed that the reactors were turning sour due to the high biodegradability of the duckweed and cow manure mixture, i.e., the acid forming bacteria rapidly produced acid resulting in low pH thereby inhibiting the methane forming bacteria or the methanogenesis process.

Re-seeding was done using sludge from a wastewater treatment plant operating at the desired temperature. It was believed that this would reduce the start up time and provide a microbial community that was already acclimated to the desired conditions. The digesters quickly reached steady state conditions after 60 days. The digesters were started on the 10/16/2009.

Digester loading

Due to the initial series of sour digesters experienced, solids loading were carried out in increments of 0.5 g and 0.25 g (dry weight basis) for the fresh and dried duckweed fed reactors, respectively. This was done to allow the microbial community to adjust to the new environment and slowly increase loading without upsetting the digesters. The only pretreatment done prior to loading was grinding of the duckweed to smaller particle sizes that could easily be fed into the reactors, while at the same time providing uniform feed stock for efficient digestion. The initial loading was 0.25 g per day and was gradually increased to 1.75 g per day after a period of 1 year. The volatile solids portion of the duckweed biomass fed into the reactors was about 70% of the total mass (Figure 6). The maximum feed load was not reached during this study period and so both digesters still have the capacity to allow loads greater than 1.75 g per day (0.875 g/L/d).

The fresh duckweed fed reactor (R 1) reached and maintained stability faster than that fed with dried duckweed (R2) biomass (Figure 9). On some occasions short

circuiting was noticed but on a small scale for both reactors. Oxygen intake was more common in R2 because the dried duckweed had a tendency to float on the water surface and clump together when being introduced to the digester.

The dried duckweed fed reactor (R2) was highly sensitive to changes in feed loading and oxygen intake but was quick to regain stability. An increase in feed led to an immediate decrease in methane production, later followed by an increase in methane before stability was attained (Figure 10). The reverse was observed in the fresh duckweed fed reactor, which showed an immediate increase in methane production later followed by a dip, then stability (Figure 11).

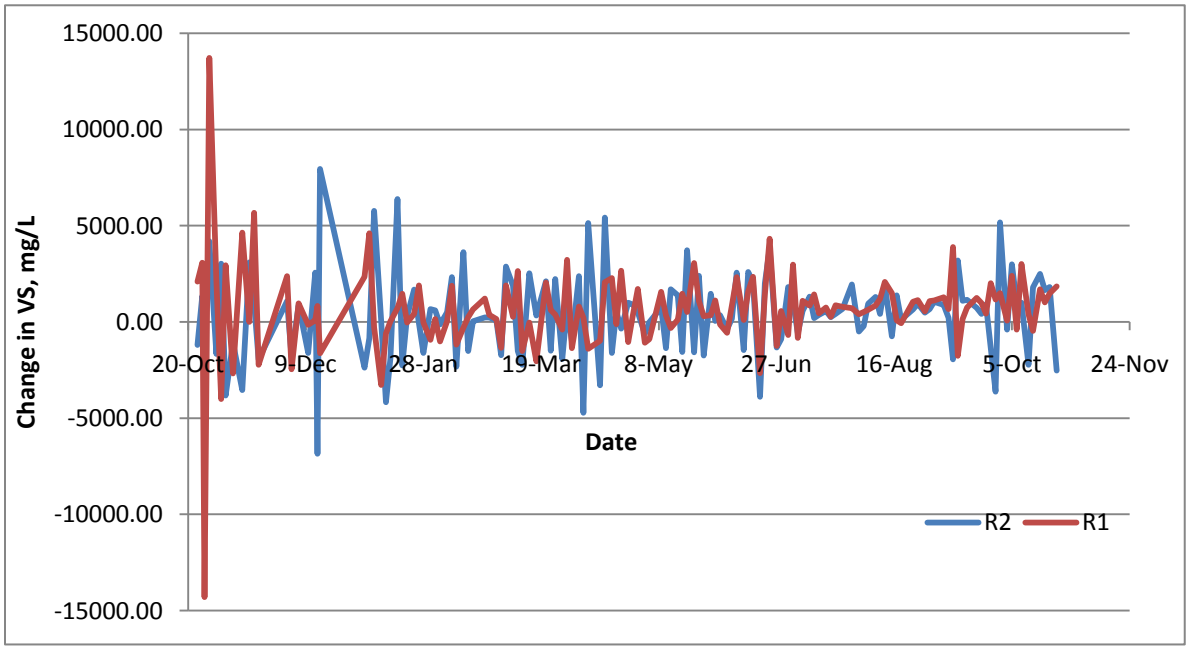

Figure 9. Daily change in VS with time in R1 and R2.

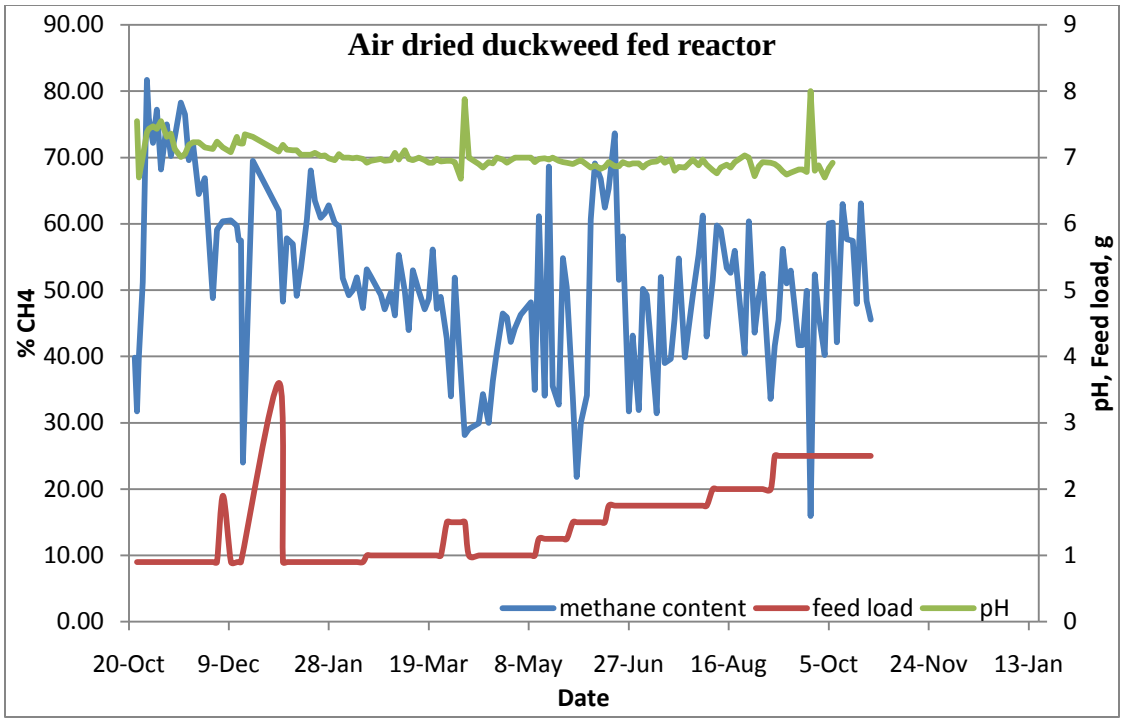

Figure 10. pH, % methane and feed variation over time for R 2.

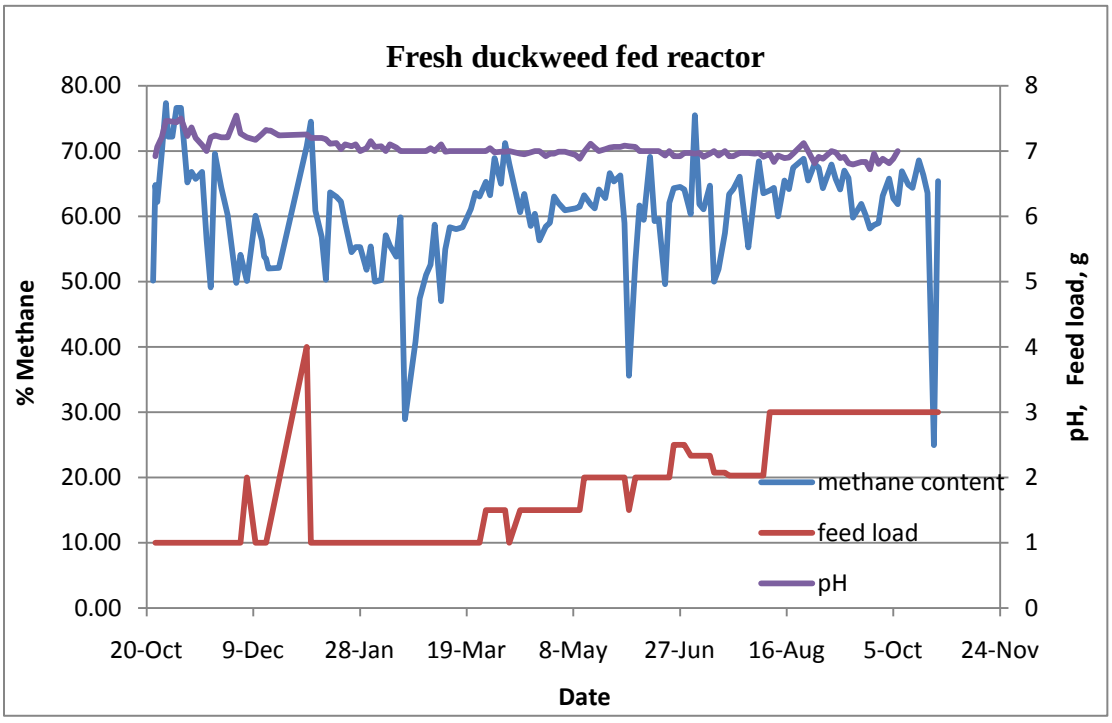

Figure 11. pH, % methane and feed variations over time for R 1.

Generally, with the increase in feed (organic matter), more feed was consumed by the bacteria resulting in increased VFA production and methane production. However, the different trends noticed for R1 and R2 after the increase in feed may have been due to the difference in buffering capacity of the two reactors (Figure 12). In addition, there was a possibility of an increased and more diverse microbial population introduced into R1 by the fresh duckweed biomass harvested from the lagoons.

The methane yield and gas composition results were as shown in Table 7. The gas composition for both reactors was above 60% methane and 30% CO_2 (Table 7). R1 showed higher methane content compared to R2. This may have been due to the fact that R1 was generally more stable than R2. The volatile solids reduction of the fermenting slurry observed was 40% and 38% for R1 and R2, respectively (Table 7). The pH conditions in both reactors were above optimum (Table 7). The methane yield from both reactors R1 and R2 was below the expected theoretical yield (Table 7), and this was an indication of the small amount of cell production occurring within the reactors, and that not all the volatile portion of the duckweed was biodegradable as confirmed by the % VS reduction values. A higher theoretical COD value of 1.36 g O_2/g VS DW was calculated compared to the measured value of 1.20 g O_2/g VS DW obtained. Also from the duckweed empirical formula, a C:N ratio of 11 was obtained which is below optimum for anaerobic digestion. The optimum C:N ranges from 20 – 30:1 for maximum gas production (Gerardi, 2003). The C:N ratio was not critical in the running of the digesters and as such no additional nutrient supplements were added.

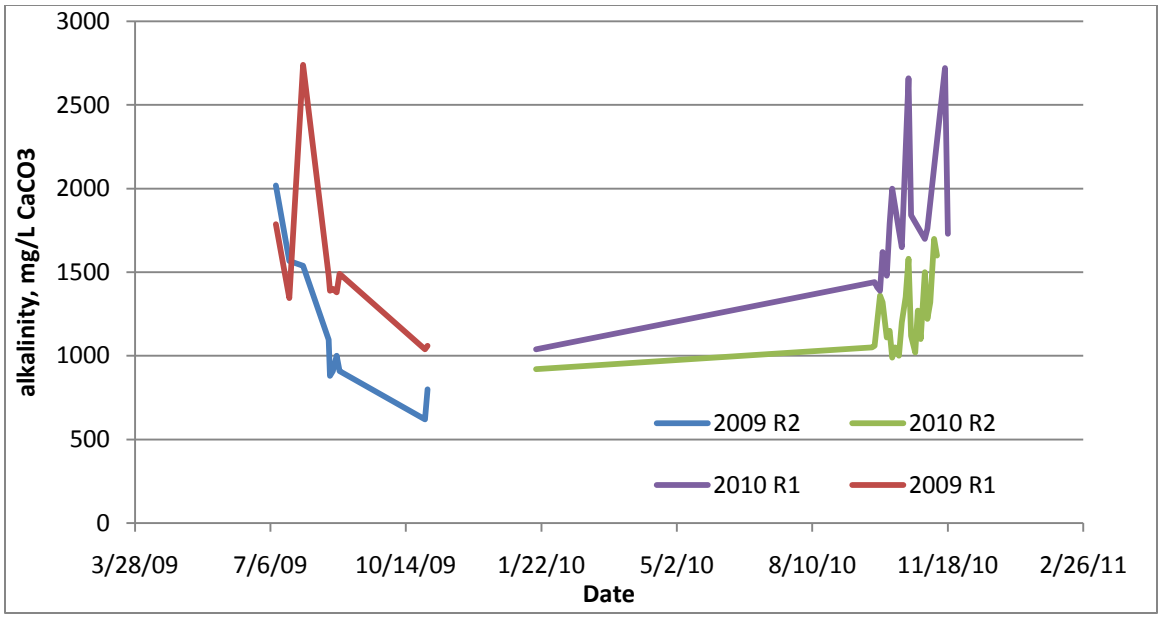

Figure 12. Alkalinity values for R1 and R2.

Table 7. Summary of the anaerobic digestion parameters for R1 and R2

Reactor name	Gas composition, %		Volume, L/kg VS destroyed		pH	% VS reduction
	CH_4	CO_2	Actual	Theoretical		
R1 (Fresh DW)	67.1 ± 1.53	32.9 ± 1.24	0.393	0.476	6.72 - 7.47	40
R2 (Air dried DW)	62.5 ± 2.37	37.5 ± 1.30	0.359	0.476	6.68 – 8.00	38

The digestibility of the duckweed biomass was determined by comparing the amount of methane COD obtained for every gram of duckweed COD (dry weight basis) fed into each of the reactors (Figure 13). The measured COD value was used instead of the theoretical value for a more accurate and realistic depiction of the reactor conditions. It was observed that the digestibility of duckweed in both R1 and R2 was the same. This was expected since the same biomass was utilized with the only difference being the physical form in which it was introduced to the reactors (dried versus fresh). An overall

conversion efficiency of the duckweed COD to methane COD over the course of the study is shown in Figure 14, and was found to be 39 and 45%for R1 and R2, respectively.

A number of VFAs were present in the digester effluent obtained from both reactors as shown in Figures 15 and 16. A difference was noticed in the distribution of VFAs at the start of digestion and after steady state conditions were attained in both reactors (Figure 15 and 16). The major acids detected at the beginning of the study were acetate, butyrate and propionate for both reactors, while lactate, acetate and propionate were the major acids identified after acclimatization. The VFA distribution at the start of the digestion was influenced by the sludge material used as the inoculum, while the steady state VFA distribution is reflective of the duckweed biomass used as the primary substrate for the balance of the study period, thus the differences noticed.

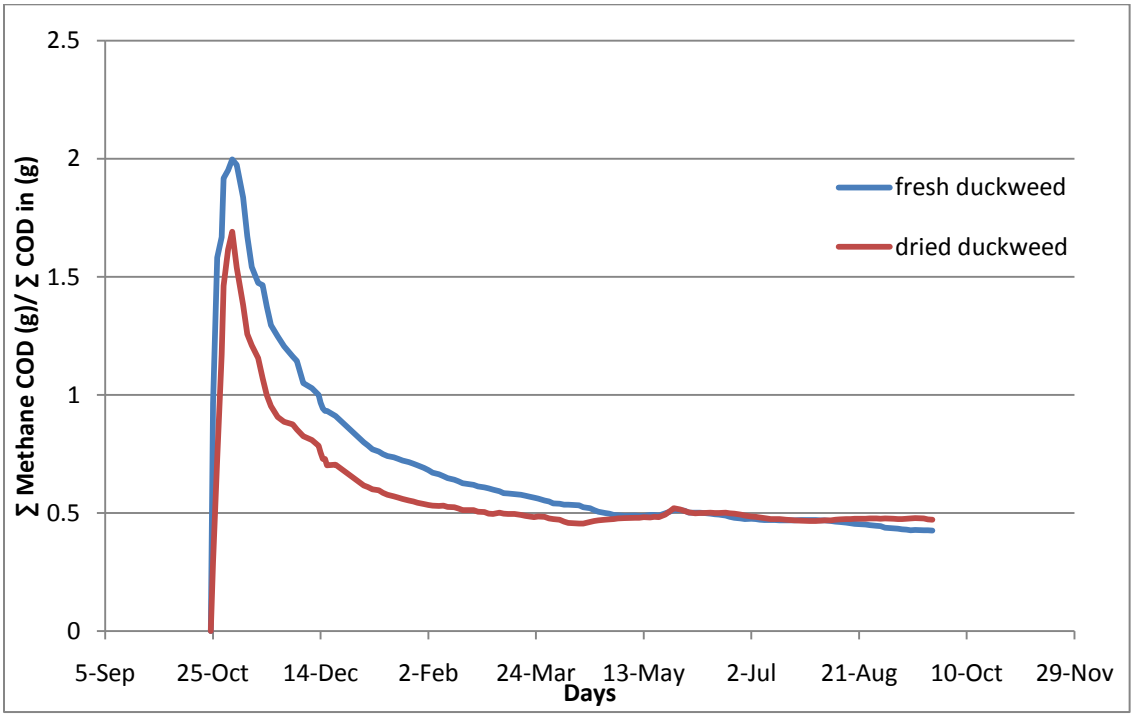

Figure 13. Digestibility of duckweed biomass per g COD.

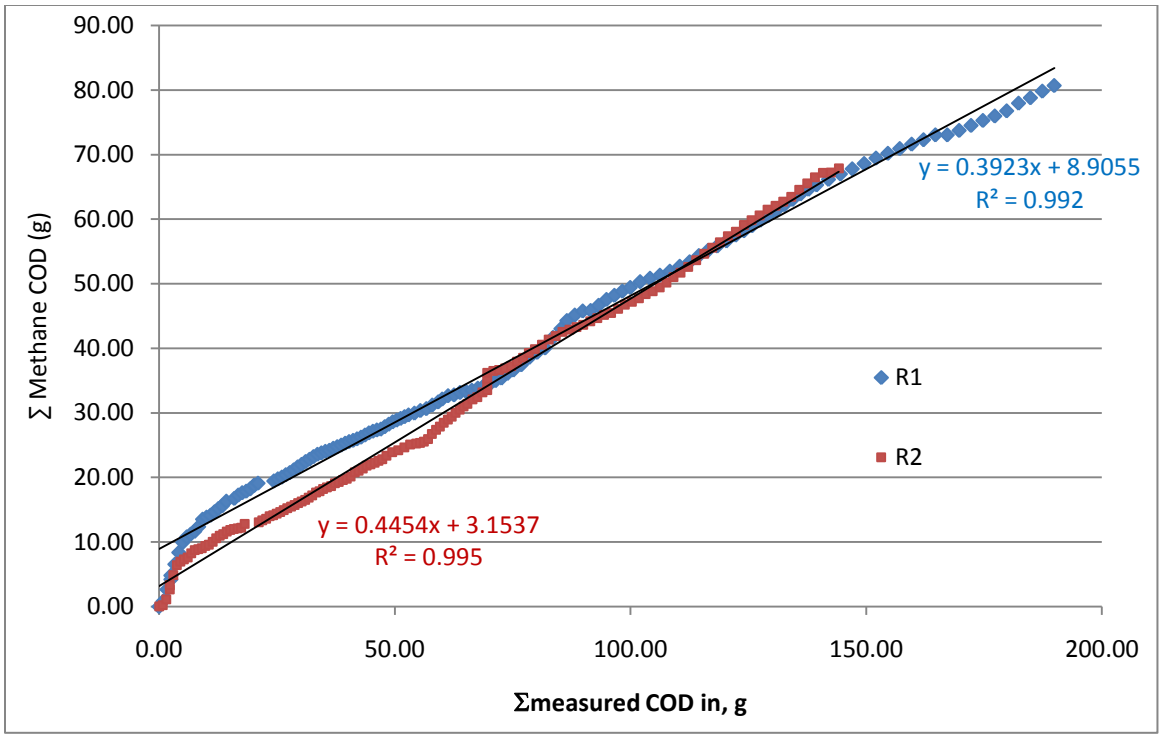

Figure 14. Conversion efficiency of duckweed COD to methane over the course of this study.

After attaining stability, the concentrations of cations in both reactors were low and not inhibitory to the digestion process as shown in Figures 17 and 18. The average NH_4^+ concentration observed for R1 and R2 was 362 mg/L and 242 mg/L, respectively.

Alkalinity values were within the acceptable range of 1000 – 3000 mg/L CaCO3, an indication of the health of the digesters (Figure 12). Lower alkalinity values were recorded for R2 compared to R1, and this may have been due to the higher concentration of VFA values (especially acetate) observed in R2 compared to R1 (Figure 15 and 16).

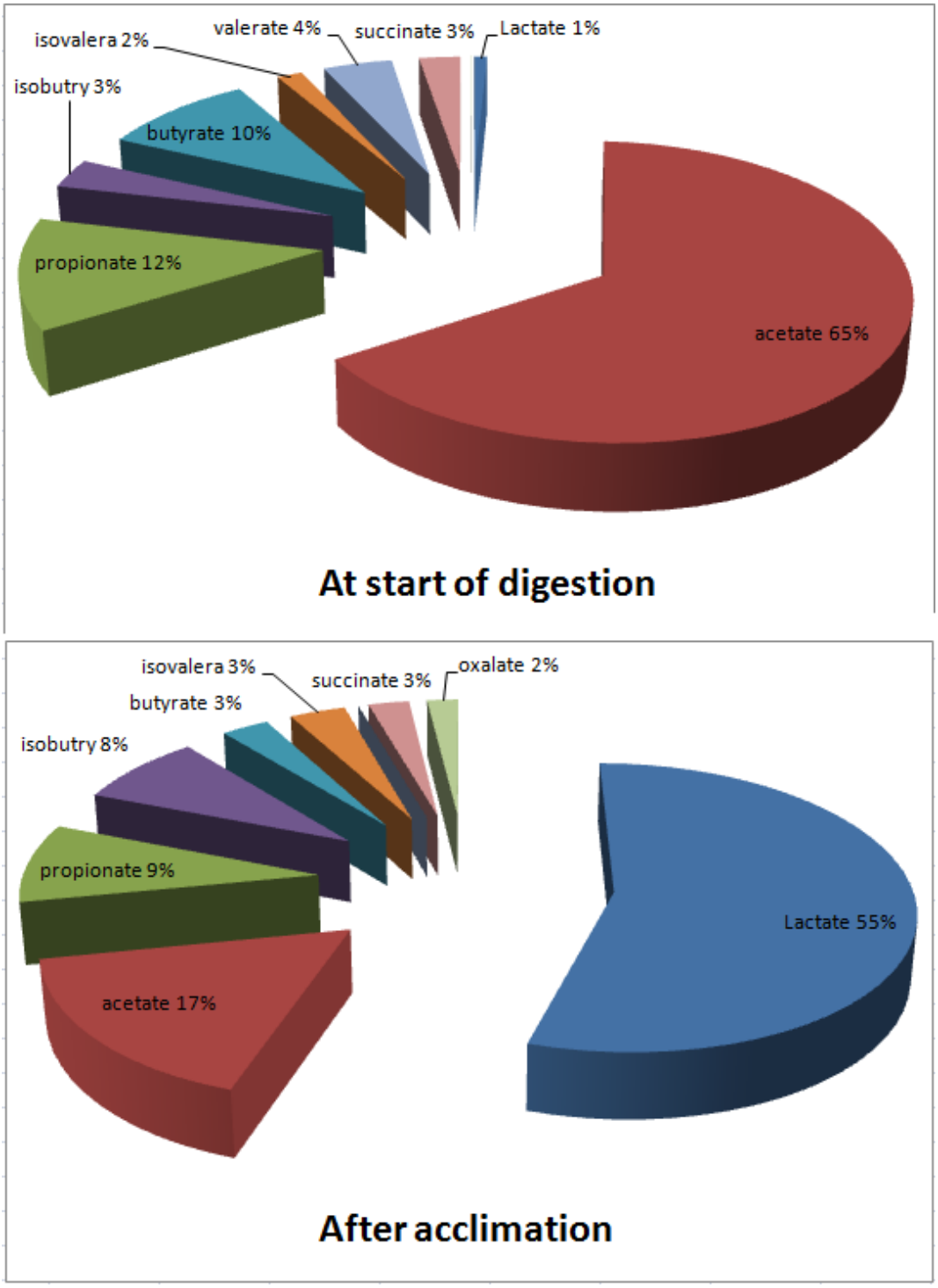

Figure 15. Effluent VFA distribution in R1.

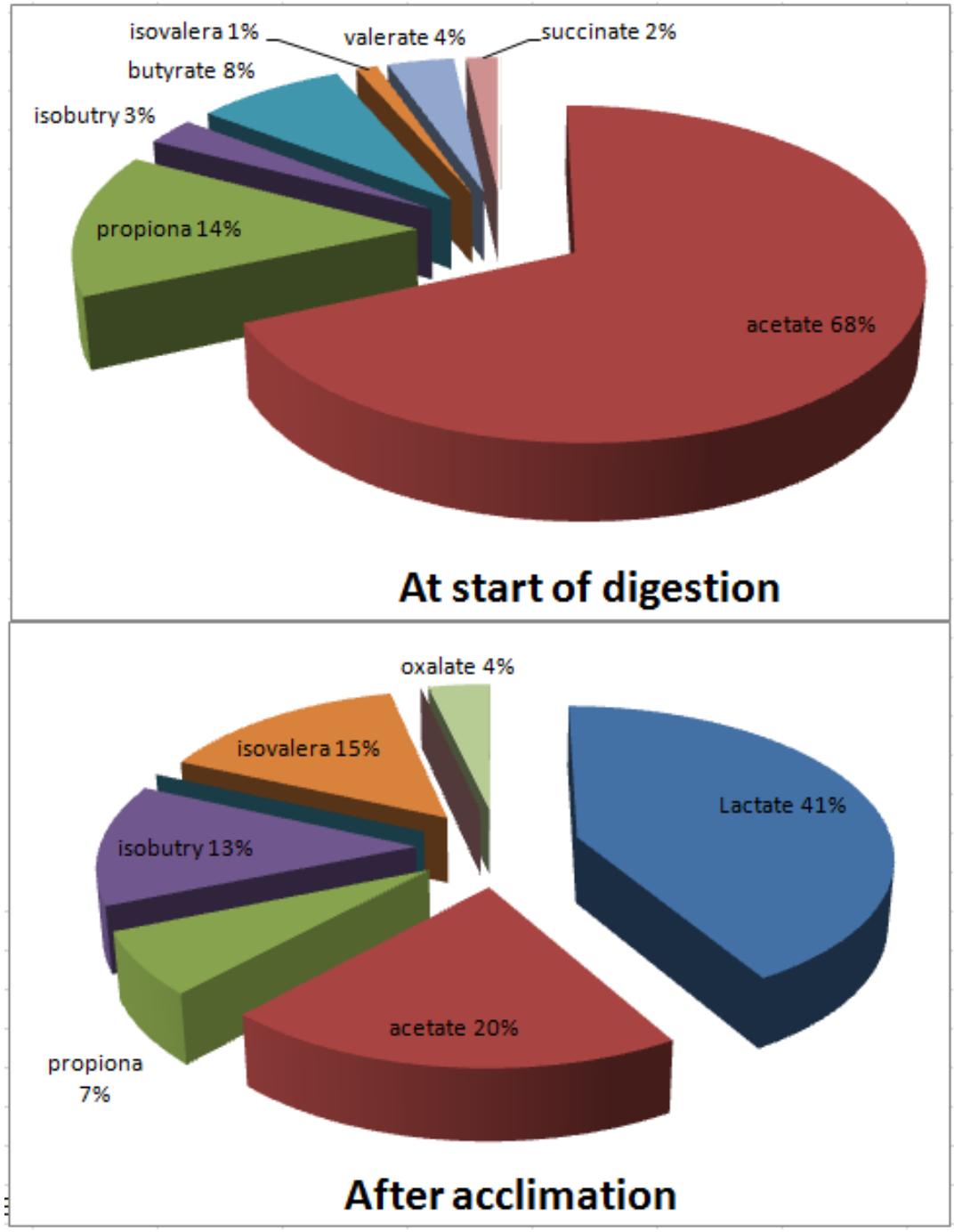

Figure 16. Effluent VFA distribution in R2.

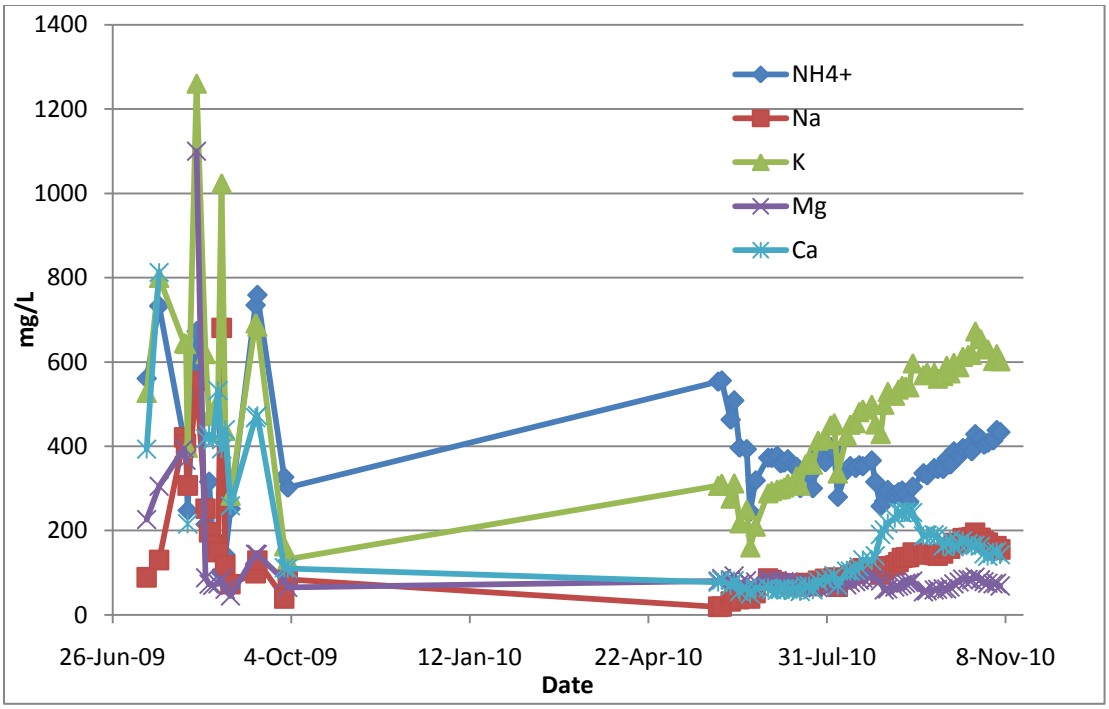

Figure 17. Effluent cation concentrations in R1.

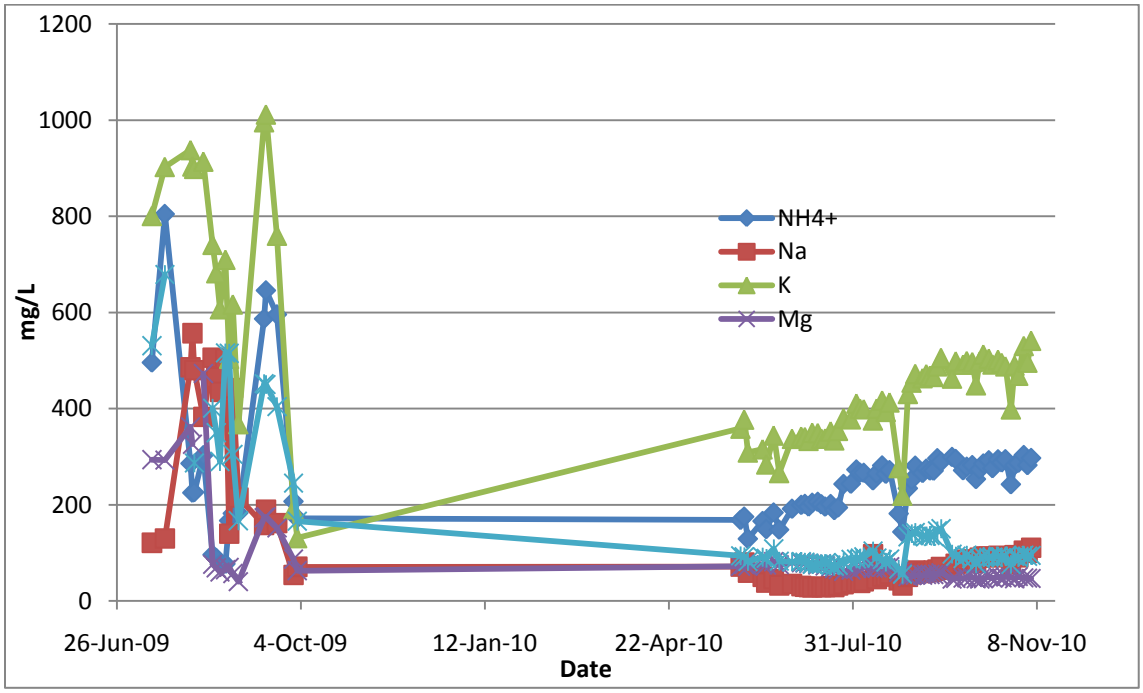

Figure 18. Effluent cation concentrations in R2.

Fermentation for Ethanol Production

Starch in duckweed

Investigations in the use of duckweed biomass for ethanol production began in Feb. 2010 and ended in Oct. 2010. The first starch measurements were obtained from oven dried duckweed biomass batch from previous growing season (summer 2009) and fresh duckweed biomass grown on wastewater solution in the lab. Oven drying was done at 103^{o}C and the duckweed material stored for use through the winter period in Ziplock bags. Low levels of starch were observed (< 10%) for both dried and fresh duckweed biomass (Table 3). An attempt was made to accumulate starch by transferring the duckweed from the wastewater solution to tap water media for 6 days. This was done to determine the amount of starch the plant would accumulate during this period. Results obtained were variable and therefore not conclusive, however, it was still noticed that starch was highest for the 6 d biomass grown on nutrient deficient solution (Figure 19).

For the 2010 duckweed growing season (late may – early November), Focus was placed on accumulating duckweed for ethanol production trials. Starch measurements were made on 6 d duckweed biomass grown on nutrient deficient Logan River water. These results were also highly variable with starch values ranging from 6-38% for fresh duckweed samples normalized to a dry weight basis (Table 6).

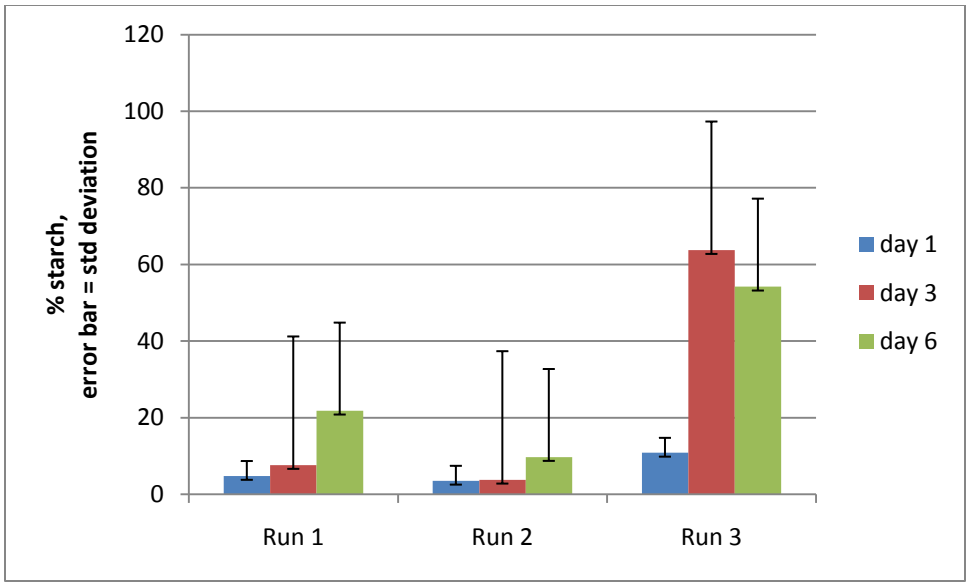

Figure 19. Starch accumulation of lab-grown duckweed plants.

The exact reason for this difference was not investigated but possibly this could have been brought about by degradation of starch due to oven drying or storage. A number of reasons were attributed to the variability and low starch values observed in the study;

i. A mixed culture of L.minor and Wolffia spp. was used in this study compared to Spirodela polyrhiza species used in the reviewed literature (Cheng and Stomp, 2009; Cui et al., 2010).

ii. The species were grown in a low strength municipal wastewater compared to swine wastewater used on previous studies (Cheng and Stomp, 2009).

iii. Duckweed growth was at ambient temperature (above 25^{o}C) which showed the least accumulation of starch in previous studies (Cui et al., 2010).

iv. Changes in the growing conditions of duckweed within the lagoons could also have been a factor since the effluent nutrient concentration changes in each lagoon cell.

v. McCombs and Ralph (1972) accumulated starch in duckweed in the dark for 6 days which was not the case in this study

Therefore, even at times when high values of starch content were realized, the values could not be replicated in proceeding runs due to the variability inherent in the sampling process and experimental design.

Ethanol production

Only 6-day old fresh duckweed, grown on nutrient deficient water, and the oven dried duckweed were utilized for the fermentation process. Expected substrate loading was 100 g DW/L but this was only possible for the oven dried duckweed and not for the fresh duckweed biomass. The limitation was due to the high moisture content in the duckweed and as such more volume would be required to achieve the desired loading. Less than 1% v/v ethanol concentration was realized for every fermentation run made (Table A-4 and A-5). A higher ethanol yield was obtained from fresh duckweed biomass compared to the dried duckweed biomass regardless of the reduced amounts used (Figure 20). This confirmed that more starch was present in fresh duckweed than in the dried duckweed. Oven drying did not prove beneficial since water had to be added to the biomass during the fermentation process and lower ethanol yields were realized. Cheng and Stomp (2009), reported an ethanol yield of 258 mg/g of dry duckweed biomass which is almost 3 times higher than the maximum ethanol yields obtained in this study (Figure 20). This was attributed to the inability to accumulate starch in the plant biomass

to levels as high as those observed by Cheng and Stomp (2009). Successful ethanol production from duckweed biomass requires determining and controlling the major factors/conditions affecting starch accumulation within the plant biomass. The ethanol production alternative for duckweed biomass from the lagoons was not feasible for this study because of the highly variable growth conditions within the lagoons. This option may be feasible if duckweed is grown in a controlled lab scale setting.

By-products resulting from both the ethanol production and anaerobic digestion process need to be properly disposed of. The phosphorus in the biomass does not disappear after either process but remains in the processed solids residue and liquid stream. Therefore, additional research is required to determine if the resulting byproducts would require further treatment before disposal. It is also important to know if there is still beneficial use for these by-products. The following section briefly illustrates this need by considering anaerobic digestion as a case study.

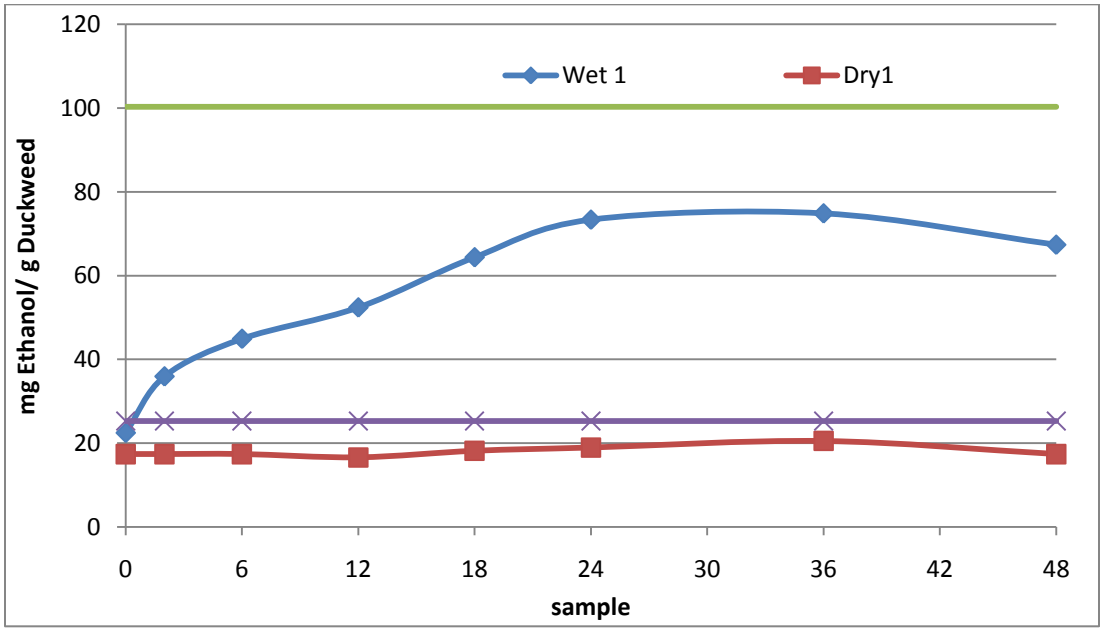

Figure 20. Ethanol yield from fresh and dried duckweed biomass.

Fate of phosphorus in the system

It is very important to keep track of the nutrients of concern (P in this case) in any kind of nutrient removal process implemented for municipal wastewaters. This acts as a check on the overall performance of the process and at the same time provides accountability. A mass balance was carried out for P for the anaerobic digestion process (Table 8). The sampling period considered for R1 and R2 was 162 and 166 days, respectively. In every time step considered, the moisture content of the feed was measured and the feed load value adjusted accordingly. The effluent volume taken out of the reactor was 100 mL/day.

The sum of P in the effluent and digested solids should equal the P coming into the system under steady state conditions. In reality, ideal steady state conditions were not achieved for R1 and R2 as shown by the percentage of the unaccounted P in the system (Table 8).

Table 8. P mass balance for the anaerobic digestion process for R1 and R2

Date (2010)		Total P into reactor			Total P out in liquid effluent		Total P out in digester solids			Unaccounted for P in reactors	
R 1	Δ Days	% P, In	Load , g/d	total P in, g/d	P, mg/L	P out, g/d	% P out	TS out, mg/L	P out, g/d	%	g P
05/14 - 06/23	41	0.0093	1.00	0.0093	50.50	0.0051	0.0084	4607.8	0.0039	3.2	0.0123
06/25 - 06/30	6	0.0093	1.25	0.0116	53.23	0.0053	0.0084	3333.7	0.0028	30.2	0.0209
07/03 - 07/12	10	0.0093	1.17	0.0108	52.49	0.0053	0.0084	5273.6	0.0044	10.2	0.0110
07/14 - 07/19	6	0.0093	1.04	0.0096	53.50	0.0054	0.0084	5690.9	0.0048	-6.3	0.0036
07/21 - 08/06	17	0.0093	1.02	0.0094	40.92	0.0041	0.0084	5891.6	0.0049	4.3	0.0068
08/09 - 10/29	82	0.0093	1.50	0.0139	28.89	0.0029	0.0084	8181.8	0.0069	29.5	0.3362
R 2											
05/14 - 05/28	15	0.0093	0.63	0.0058	45.15	0.0045	0.0092	3080.8	0.0026	-22.4	0.0195
05/31 - 06/16	17	0.0093	0.75	0.007	41.80	0.0042	0.0092	4157.6	0.0035	-10.1	0.0119
06/18 - 08/06	50	0.0093	0.88	0.0081	38.38	0.0038	0.0092	4848.1	0.0041	2.5	0.0100
08/09 - 09/07	30	0.0093	1.00	0.0093	39.44	0.0039	0.0092	5361.2	0.0045	9.7	0.0270
09/19 - 11/01	54	0.0093	1.25	0.0116	34.25	0.0034	0.0092	6152.4	0.0052	25.9	0.1620

The average P fed into the reactors for the time period considered was 1918.57 and 1517.05 mg P for R1 and R2, respectively. Average P taken out in the liquid effluent was 633.62 and 637.33 mg P for R1 and R2, respectively, and average P taken out in the digested solids was 893.53 and 711.62 mg P, respectively. Therefore, the recovery efficiency obtained for R1 and R2 was 79.6% and 88.9%, respectively. The difference in P recovery efficiency for R1 and R2 may have been due to: insufficient effluent liquid and solids P measurements taken, and difference in digestibility between the fresh and dried duckweed samples. In order to accurately measure P in the solids, the samples had to be digested as indicated in the method used (Appendix D). The percent unaccounted P values are an indication of accumulation of solids (+ % values) or less feed (- % values) into the reactor than that reported (Table 8).

The average P concentration observed in the effluent stream for R1 and R2 was 38 ± 13 mg/L and 34 ± 8 mg/L (Table 9), respectively. Returning such a concentrated P stream into the lagoons undermines the treatment process. Therefore there is a need to harvest P in the effluent stream if this process is to be successful. Precipitation of P into struvite ($MgNH_4PO_4*6H_2O$) that is used as a fertilizer is one option that can be undertaken. Rahaman, Ellis and Mavinic, (2008) reported higher P removal efficiency via struvite precipitation with increasing Mg:P ratio with the optimum ratio occurring between 1.1 – 1.6. The Mg:P obtained for the effluent from R1 and R2 were 2 and 1.8, respectively (Table 9). Further research on this process is recommended.

The digested biosolids retain the largest fraction of P compared to the liquid effluent. These biosolids should therefore be properly disposed of. The potential feed value from the digested biosolids was investigated (Figure 21). It was observed that the

digested solids contained higher NDF and ADF values compared to the original duckweed biomass and its CP values were lower than those observed for the original biomass. Overall, the RFV values obtained for the digested biomass were 130.7 and 151.7 for air-dried and fresh DW, categorizing them as fair and good feed, respectively (Table A- 4 and C-1).

Table 9. Average nutrient concentrations (mg/L) in the digester effluent for R1 and R2

Name	Na+	NH4+	K+	Mg2+	Ca2+	PO4-P	NO3-N	Mg : P
R1	109.23	362.71	456.35	74.60	128.91	38.06	49.28	1.96
R2	60.72	242.57	414.33	61.12	94.24	34.18	37.06	1.79

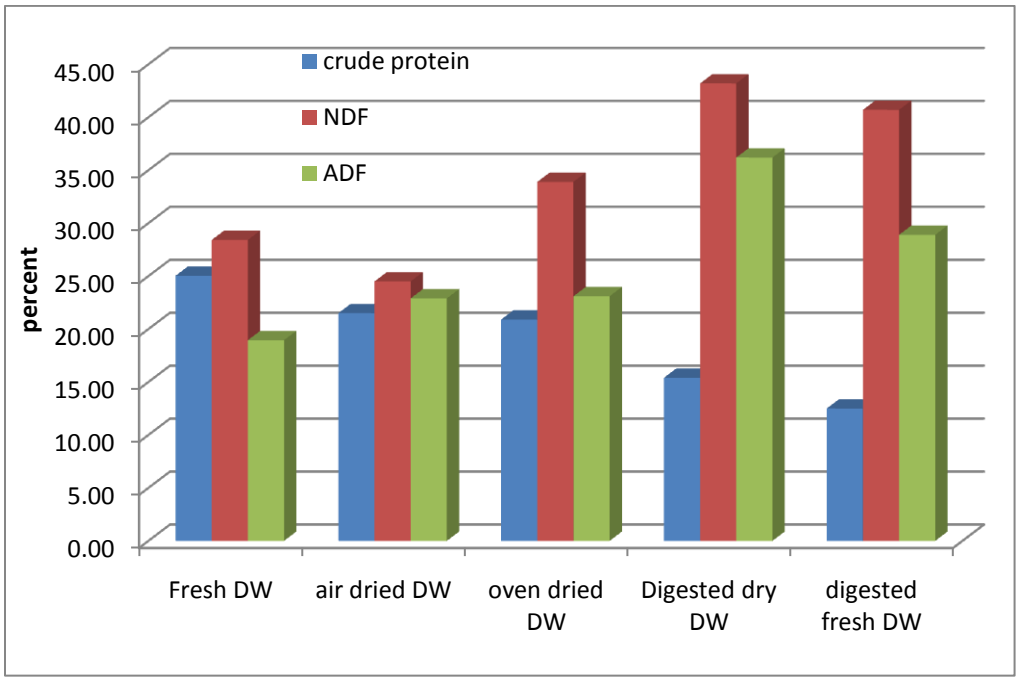

Figure 21. Comparison of feed quality of the digested biosolids to the undigested duckweed biomass.

CONCLUSIONS

i. Duckweed biomass reuse is possible and should be considered if duckweed is incorporated into the Wellsville City wastewater treatment system. The only biomass preprocessing that was required in this study was grinding into smaller uniform sized particles for fresh and dried duckweed.

ii. Anaerobic digestion and animal feed options were the viable biomass management techniques that emerged from this study.

iii. The recommended duckweed biomass management option for a small community like Wellsville is anaerobic digestion because it is a source of energy and at the same time the digestate can be used as a low quality feed or a soil conditioner, and the digester liquid could serve as a feedstock for recovery of N and P via struvite precipitation.

ENGINEERING SIGNIFICANCE

In the context of the this study, there is concern regarding increased nutrient loading into the Little Bear River by discharge from the Wellsville City Sewage lagoons resulting in an impairment for its beneficial use. The use of duckweed as a nutrient removal option for the lagoon systems can only be realized through regular duckweed harvesting, which will result in a nutrient rich biomass byproduct. This is an inexpensive nutrient removal option that will help the lagoons meet their discharge limits while at the same time providing a safe and clean water source to the community and the receiving water. The importance of this research therefore is to help realize this goal by evaluating alternatives to processing, management and disposal or reuse of the harvested biomass in order to make the system sustainable and economically feasible for the community.

FUTURE STUDIES

1. It is recommended that if fresh duckweed is to be utilized for anaerobic digestion, fermentation for ethanol production, or as an animal feed, it should be dewatered to reduce the reactor volume requirements, and solids handling and transportation costs.
2. Further research on accumulation of starch on the *Lemna minor* and *Wolffia* species in a controlled experimental design could prove beneficial, as use of the biomass for ethanol production will only be beneficial if substantial amounts of starch (> 40%) are accumulated in the biomass.
3. Further research should be carried out on the animal feed option, especially regarding how to balance the plant nutrients in a feed.
4. It is also recommended that if duckweed is to be used as animal feed the source and composition of the wastewater on which it is grown be known. Duckweed grown on wastewater with high concentrations of heavy metals, for example, may bioaccumulate in the duckweed and subsequently should not be fed to animals. Further evaluation of the bioaccumulation potential of hazardous organics and metals within duckweed is warranted.
5. It is has been suggested that pathogen transmission from wastewater grown duckweed to ruminants can be minimized by providing a sufficient retention time in clean water. The exact time required for this was not provided and thus there is need for further research on this topic as well.
6. Since the growth of duckweed depends on the season, either co-digestion or utilizing both fresh and dried duckweed biomass as a feedstock should be

considered. A schedule should be evaluated where fresh duckweed is fed in the summer months, while dried duckweed is gradually added at the end of the summer growing season and then would be the sole feed source in the winter. With this in mind, optimal drying and storage techniques should be investigated.

7. Further research should be done on the feasibility of struvite harvesting from the anaerobic digester effluent.
8. Additional research is required to determine if the byproducts from any of the alternatives chosen would require further treatment before disposal or if they can be used for other beneficial purposes.

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APPENDICES

APPENDIX A: FEED REPORTS AND LAB RESULTS

Results from the USU Analytical Lab, USU Skaggs's Lab, the Huffman Lab and UWRL lab

Feed Report

USU Analytical Labs
Utah State University
Logan, Utah 84322-4830
(435) 797-2217
(435) 797-2117 (FAX)

Date Received 8/28/2008
Date Completed 9/10/2008
Name RYAN DUPONT
Address:
UWRL 8200
Lab Number: 8020095
Identification: SOUTH LAGOON 1 126480
Feed Material: RFV: 217

		AS SAMPLED:	DRY MATTER BASI
Moisture	%	10.8	
Dry Matter	%	89.2	100
Protein	%	24.8	27.8
ADF	%	21.7	24.3
NDF	%	30.9	34.6
Unavailable Protein	%		
Ash	%		
Nitrate-Nitrogen	mg/kg		
Total Digestible Nutrients – TDN	%	59.2	66.4
Net Energy Lactation – NEL	Mcal/lb	0.61	0.68
Net Energy Maintenance – NEM	Mcal/lb	0.61	0.69
Net Energy Gain – NEG	Mcal/lb	0.37	0.42
MINERALS:			
Calcium	%	5.63	6.31
Phosphorus	%	0.88	0.98
Potassium	%	3.03	3.39
Magnesium	%	0.41	0.46
Sodium	mg/kg	8510	9540
Sulfur	%	0.65	0.73
Aluminum	mg/kg	2312	2591
Boron	mg/kg	549.0	615.5
Cadmium	mg/kg	0.4	0.5
Cobalt	mg/kg	0.4	0.5
Chromium	mg/kg	4.8	5.4
Copper	mg/kg	59.3	66.5
Iron	mg/kg	966.5	1083.5
Manganese	mg/kg	138.4	155.1
Molybdenum	mg/kg	0.0	0.0
Nickel	mg/kg	20	23
Lead	mg/kg	6	7
Strontium	mg/kg	212.0	237.6
Zinc	mg/kg	156.7	175.7
Arsenic	mg/kg		
Selenium	mg/kg		

NOTES:
- The sampling technique used to obtain this sample will determine if the sample represents the lot.
- A value of "0" indicates that the value was below detection limits.
-Arsenic and selenium analyses performed by ICP-mass spec at the USU Animal Diagnostic Lab.
-Nitrate-Nitrogen values <1100 mg/kg - should be safe for all animals.
>1100mg/kg but <2300 mg/kg - use caution; dilute with other feeds; do not feed to pregnant animals.
>2300 mg/kg - DO NOT FEED!

Feed Report

USU Analytical Labs
Utah State University
Logan, Utah 84322-4830
(435) 797-2217
(435) 797-2117 (FAX)

Date Received 8/28/2008
Date Completed 9/10/2008
Name RYAN DUPONT
Address:
UWRL 8200
Lab Number: 8020096
Identification: NORTH LAGOON 1 126479
Feed Material: RFV: 291

		AS SAMPLED:	DRY MATTER BASIS:
Moisture	%	6.1	
Dry Matter	%	93.9	100
Protein	%	36.1	38.4
ADF	%	15.9	16.9
NDF	%	24.5	26.1
Unavailable Protein	%		
Ash	%		
Nitrate-Nitrogen	mg/kg		
Total Digestible Nutrients – TDN	%	2.6	2.80
Net Energy Lactation – NEL	Mcal/lb	0.75	0.80
Net Energy Maintenance – NEM	Mcal/lb	0.78	0.83
Net Energy Gain – NEG	Mcal/lb	0.51	0.55
MINERALS:			
Calcium	%	3.29	3.50
Phosphorus	%	0.78	0.83
Potassium	%	3.26	3.47
Magnesium	%	0.36	0.38
Sodium	mg/kg	11150	11874
Sulfur	%	0.58	0.62
Aluminum	mg/kg	445	474
Boron	mg/kg	630.5	671.5
Cadmium	mg/kg	0.2	0.2
Cobalt	mg/kg	0.0	0.0
Chromium	mg/kg	1.2	1.3
Copper	mg/kg	40.9	43.5
Iron	mg/kg	401.3	427.3
Manganese	mg/kg	162.2	172.7
Molybdenum	mg/kg	0.0	0.0
Nickel	mg/kg	2	2
Lead	mg/kg	5	5
Strontium	mg/kg	128.1	136.4
Zinc	mg/kg	69.2	73.6
Arsenic	mg/kg		
Selenium	mg/kg		

NOTES:
- The sampling technique used to obtain this sample will determine if the sample represents the lot.
- A value of "0" indicates that the value was below detection limits.
-Arsenic and selenium analyses performed by ICP-mass spec at the USU Animal Diagnostic Lab.
-Nitrate-Nitrogen values <1100 mg/kg - should be safe for all animals.
>1100mg/kg but <2300 mg/kg - use caution; dilute with other feeds; do not feed to pregnant animals.
>2300 mg/kg - DO NOT FEED!

Feed Report

USU Analytical Labs
Utah State University
Logan, Utah 84322-4830
(435) 797-2217
(435) 797-2117 (FAX)

Date Received 8/28/2008
Date Completed 9/10/2008
Name RYAN DUPONT
Address:
UWRL 8200
Lab Number: S020097
Identification: SOUTH LAGOON 3 126474
Feed Material: RFV: 245

		AS SAMPLED:	DRY MATTER BASIS
Moisture	%	7.9	
Dry Matter	%	92.1	100
Protein	%	19.7	21.4
ADF	%	18.4	20.0
NDF	%	28.3	30.7
Unavailable Protein	%		
Ash	%		
Nitrate-Nitrogen	mg/kg		
Total Digestible Nutrients – TDN	%	58.5	63.5
Net Energy Lactation – NEL	Mcal/lb	0.60	0.65
Net Energy Maintenance – NEM	Mcal/lb	0.59	0.65
Net Energy Gain – NEG	Mcal/lb	0.35	0.38
MINERALS:			
Calcium	%	6.12	6.64
Phosphorus	%	1.14	1.23
Potassium	%	4.46	4.84
Magnesium	%	0.55	0.60
Sodium	mg/kg	6750	7329
Sulfur	%	0.75	0.81
Aluminum	mg/kg	144	157
Boron	mg/kg	843.0	915.3
Cadmium	mg/kg	0.1	0.1
Cobalt	mg/kg	0.3	0.3
Chromium	mg/kg	1.1	1.2
Copper	mg/kg	9.3	10.1
Iron	mg/kg	214.2	232.6
Manganese	mg/kg	251.0	272.5
Molybdenum	mg/kg	0.0	0.0
Nickel	mg/kg	1	1
Lead	mg/kg	0	0
Strontium	mg/kg	223.9	243.1
Zinc	mg/kg	14.3	15.5
Arsenic	mg/kg		
Selenium	mg/kg		

NOTES:
- The sampling technique used to obtain this sample will determine if the sample represents the lot.
- A value of "0" indicates that the value was below detection limits.
-Arsenic and selenium analyses performed by ICP-mass spec at the USU Animal Diagnostic Lab.
-Nitrate-Nitrogen values <1100 mg/kg - should be safe for all animals.
>1100mg/kg but <2300 mg/kg - use caution; dilute with other feeds; do not feed to pregnant animals.
>2300 mg/kg - DO NOT FEED!

Feed Report

USU Analytical Labs
Utah State University
Logan, Utah 84322-4830
(435) 797-2217
(435) 797-2117 (FAX)

Date Received 8/28/2008
Date Completed 9/10/2008
Name RYAN DUPONT
Address:
UWRL 8200
Lab Number: 8020098
Identification: SOUTH LAGOON 4 126476
Feed Material: RFV: 265

		AS SAMPLED:	DRY MATTER BASIS:
Moisture	%	8.1	
Dry Matter	%	91.9	100
Protein	%	20.0	21.8
ADF	%	18.8	20.5
NDF	%	26.1	28.4
Unavailable Protein	%		
Ash	%		
Nitrate-Nitrogen	mg/kg		
Total Digestible Nutrients – TDN	%	58.4	63.6
Net Energy Lactation – NEL	Mcal/lb	0.60	0.65
Net Energy Maintenance – NEM	Mcal/lb	0.60	0.65
Net Energy Gain – NEG	Mcal/lb	0.35	0.38
MINERALS:			
Calcium	%	6.53	7.11
Phosphorus	%	1.01	1.10
Potassium	%	4.07	4.43
Magnesium	%	0.50	0.54
Sodium	mg/kg	4491	4887
Sulfur	%	0.68	0.74
Aluminum	mg/kg	192	209
Boron	mg/kg	600.5	653.4
Cadmium	mg/kg	0.1	0.1
Cobalt	mg/kg	0.4	0.4
Chromium	mg/kg	1.0	1.0
Copper	mg/kg	5.1	5.6
Iron	mg/kg	275.8	300.1
Manganese	mg/kg	309.8	337.1
Molybdenum	mg/kg	0.0	0.0
Nickel	mg/kg	1	1
Lead	mg/kg	0	0
Strontium	mg/kg	194.0	211.0
Zinc	mg/kg	11.1	12.1
Arsenic	mg/kg		
Selenium	mg/kg		

NOTES:
- The sampling technique used to obtain this sample will determine if the sample represents the lot.
- A value of "0" indicates that the value was below detection limits.

CUSTOMER #: 05262

DATE 9/15/08

LAB# 167408

P.O. CREDIT CRD

RECD 08/29/08

ANALYSIS REPORT

RYAN R. DUPONT

UT STATE UNIV/WATER RES

8200 OLD MAIN HILL

LOGAN UT 84322

SEQUENCE /	01	02	03	04
SAMPLE ID	1. SOUTH LAGOON	2. NORTH EAST	3. SOUTH LAGOON	4. SOUTH LAGOON
Drying Loss---%	7.92	9.27	10.34	9.90
Carbon-----%	40.32	36.77	35.31	34.89
Hydrogen ----%	5.30	4.78	4.52	4.53
Nitrogen-----%	5.15	4.15	3.22	3.37
Oxygen (diff)-%	29.45	27.06	29.36	29.36
Sulfur-----%	0.74	0.76	0.90	0.84
Ash---------%	19.04	26.48	26.69	27.01

Full Sample No. for Sequence No. 01 is: 1. SOUTH LAGOON 1 UWRL #126480

Full Sample No. for Sequence No. 02 is: 2. NORTH EAST LAGOON UWRL

Full Sample No. for Sequence No. 03 is: 3. SOUTH LAGOON 3 UWRL #126474

Full Sample No. for Sequence No. 04 is: 4. SOUTH LAGOON 4 UWRL #126476

The samples were ground prior to analysis.

Loss on drying was determined in air at 105 degrees C overnight and is reported on an as received sample basis. All other results are reported on a dried sample basis.

Feed Report

USU Analytical Labs
Utah State University
Logan, Utah 84322-4830
(435) 797-2217
(435) 797-2117 (FAX)

2007 Certified Chemistry by NFTA — Excellence in Forage Testing — National Forage Testing Association — DM CP ADF NDF

Date Received 10/6/2010
Date Completed 11/2/2010

Name Maureen Kesaano
Address: DR. RYAN DUPONT
1600 CANYON RD
LOGAN UT 84341 CACHE

Lab Number: 1002-5291
Identification: Digested Dried Duckweed
Feed Material: Duckweed

		AS SAMPLED:	DRY MATTER BASIS:
Moisture	%	95.0	
Dry Matter	%	5.0	100
Protein	%	0.77	15.4
ADF	%	1.81	36.2
NDF	%	2.16	43.2
Unavailable Protein	%		
Ash	%		
Nitrate-Nitrogen	mg/kg		
Total Digestible Nutrients - TDN	%		
Net Energy Lactation - NEL	Mcal/lb		
Net Energy Maintenance - NEM	Mcal/lb		
Net Energy Gain - NEG	Mcal/lb		
MINERALS:			
Calcium	%	0.49	9.74
Phosphorus	%	0.04	0.79
Potassium	%	0.03	0.64
Magnesium	%	0.02	0.41
Sodium	mg/kg	62	1247
Sulfur	%	0.01	0.26
Aluminum	mg/kg	11	228
Boron	mg/kg	49.7	994.6
Cadmium	mg/kg	0.0	0.2
Cobalt	mg/kg	0.0	0.4
Chromium	mg/kg	0.0	0.9
Copper	mg/kg	1.3	26.7
Iron	mg/kg	11.6	231.0
Manganese	mg/kg	17.6	352.8
Molybdendum	mg/kg	0.0	0.0
Nickel	mg/kg	0	2
Lead	mg/kg	0	3
Strontium	mg/kg	10.8	215.9
Zinc	mg/kg	11.7	233.5
Arsenic	mg/kg		
Selenium	mg/kg		

NOTES:

- The sampling technique used to obtain this sample will determine if the sample represents the lot.
- A value of "0" indicates that the value was below detection limits.
- Arsenic and selenium analyses performed by ICP-mass spec at the USU Animal Diagnostic Lab.
- Nitrate-Nitrogen values < 1100 mg/kg - should be safe for all animals.
  >1100mg/kg but <2300 mg/kg - use caution; dilute with other feeds; do not feed to pregnant animals.
  >2300 mg/kg - DO NOT FEED!

Feed Report

USU Analytical Labs
Utah State University
Logan, Utah 84322-4830
(435) 797-2217
(435) 797-2117 (FAX)

2007 Certified Chemistry by NFTA — National Forage Testing Association — Excellence in Forage Testing — DM CP ADF NDF

Date Received 10/6/2010
Date Completed 11/2/2010

Name Maureen Kesaano
Address: DR. RYAN DUPONT
1600 CANYON RD
LOGAN UT 84341 CACHE

Lab Number: 1002-5292
Identification: Digested Fresh Duckweed
Feed Material: Duckweed

		AS SAMPLED:	DRY MATTER BASIS:
Moisture	%	94.3	
Dry Matter	%	5.7	100
Protein	%	0.71	12.5
ADF	%	1.65	28.9
NDF	%	2.32	40.7
Unavailable Protein	%		
Ash	%		
Nitrate-Nitrogen	mg/kg		
Total Digestible Nutrients - TDN	%		
Net Energy Lactation - NEL	Mcal/lb		
Net Energy Maintenance - NEM	Mcal/lb		
Net Energy Gain - NEG	Mcal/lb		
MINERALS:			
Calcium	%	0.49	8.51
Phosphorus	%	0.04	0.64
Potassium	%	0.06	1.02
Magnesium	%	0.03	0.47
Sodium	mg/kg	156	2730
Sulfur	%	0.01	0.26
Aluminum	mg/kg	35	620
Boron	mg/kg	43.8	768.0
Cadmium	mg/kg	0.0	0.1
Cobalt	mg/kg	0.0	0.5
Chromium	mg/kg	0.1	1.9
Copper	mg/kg	3.2	56.1
Iron	mg/kg	18.5	324.0
Manganese	mg/kg	19.6	343.0
Molybdendum	mg/kg	0.0	0.0
Nickel	mg/kg	0	2
Lead	mg/kg	0	2
Strontium	mg/kg	9.5	167.0
Zinc	mg/kg	19.7	345.0
Arsenic	mg/kg		
Selenium	mg/kg		

NOTES:

- The sampling technique used to obtain this sample will determine if the sample represents the lot.
- A value of "0" indicates that the value was below detection limits.
-Arsenic and selenium analyses performed by ICP-mass spec at the USU Animal Diagnostic Lab.

-Nitrate-Nitrogen values < 1100 mg/kg - should be safe for all animals.
>1100mg/kg but <2300 mg/kg - use caution; dilute with other feeds; do not feed to pregnant animals.
>2300 mg/kg - DO NOT FEED!

Feed Report

USU Analytical Labs
Utah State University
Logan, Utah 84322-4830
(435) 797-2217
(435) 797-2117 (FAX)

NATIONAL FORAGE TESTING ASSOCIATION
2007 Certified Chemistry by NFTA
Excellence in Forage Testing
DM CP ADF NDF

Date Received 10/6/2010
Date Completed 11/2/2010

Name Maureen Kesaano
Address: DR. RYAN DUPONT
1600 CANYON RD
LOGAN UT 84341

CACHE

Lab Number: 1002-5293
Identification: Air Dried Duckweed
Feed Material: Duckweed

		AS SAMPLED:	DRY MATTER BASIS:
Moisture	%	5.3	
Dry Matter	%	94.7	100
Protein	%	20.4	21.5
ADF	%	21.7	22.9
NDF	%	23.2	24.5
Unavailable Protein	%		
Ash	%		
Nitrate-Nitrogen	mg/kg		
Total Digestible Nutrients - TDN	%		
Net Energy Lactation - NEL	Mcal/lb		
Net Energy Maintenance - NEM	Mcal/lb		
Net Energy Gain - NEG	Mcal/lb		
MINERALS:			
Calcium	%	9.12	9.63
Phosphorus	%	0.66	0.70
Potassium	%	2.99	3.16
Magnesium	%	0.73	0.77
Sodium	mg/kg	6810	7191
Sulfur	%	0.46	0.48
Aluminum	mg/kg	198	209
Boron	mg/kg	536.0	566.0
Cadmium	mg/kg	0.0	0.0
Cobalt	mg/kg	0.0	0.0
Chromium	mg/kg	0.7	0.8
Copper	mg/kg	14.4	15.2
Iron	mg/kg	205.0	216.5
Manganese	mg/kg	249.0	262.9
Molybdendum	mg/kg	0.0	0.0
Nickel	mg/kg	1	1
Lead	mg/kg	2	3
Strontium	mg/kg	204.0	215.4
Zinc	mg/kg	153.0	161.6
Arsenic	mg/kg		
Selenium	mg/kg		

NOTES:

- The sampling technique used to obtain this sample will determine if the sample represents the lot.
- A value of "0" indicates that the value was below detection limits.
-Arsenic and selenium analyses performed by ICP-mass spec at the USU Animal Diagnostic Lab.
-Nitrate-Nitrogen values < 1100 mg/kg - should be safe for all animals.

Feed Report

USU Analytical Labs
Utah State University
Logan, Utah 84322-4830
(435) 797-2217
(435) 797-2117 (FAX)

2007 Certified Chemistry by NFTA — National Forage Testing Association — Excellence in Forage Testing — DM CP ADF NDF

Date Received 10/6/2010
Date Completed 11/2/2010

Name Maureen Kesaano
Address: DR. RYAN DUPONT
1600 CANYON RD
LOGAN UT 84341 CACHE

Lab Number: 1002-5294
Identification: Oven Dried Duckweed
Feed Material: Duckweed

		AS SAMPLED:	DRY MATTER BASIS:
Moisture	%	4.4	
Dry Matter	%	95.6	100
Protein	%	20.0	20.9
ADF	%	22.1	23.1
NDF	%	32.4	33.9
Unavailable Protein	%		
Ash	%		
Nitrate-Nitrogen	mg/kg		
Total Digestible Nutrients - TDN	%		
Net Energy Lactation - NEL	Mcal/lb		
Net Energy Maintenance - NEM	Mcal/lb		
Net Energy Gain - NEG	Mcal/lb		
MINERALS:			
Calcium	%	5.97	6.24
Phosphorus	%	0.91	0.96
Potassium	%	4.51	4.72
Magnesium	%	0.46	0.48
Sodium	mg/kg	9900	10356
Sulfur	%	0.58	0.61
Aluminum	mg/kg	140	146
Boron	mg/kg	827.0	865.1
Cadmium	mg/kg	0.0	0.0
Cobalt	mg/kg	0.0	0.0
Chromium	mg/kg	1.2	1.2
Copper	mg/kg	9.1	9.5
Iron	mg/kg	192.0	200.8
Manganese	mg/kg	323.0	337.9
Molybdendum	mg/kg	0.0	0.0
Nickel	mg/kg	1	1
Lead	mg/kg	0	0
Strontium	mg/kg	152.0	159.0
Zinc	mg/kg	39.4	41.2
Arsenic	mg/kg		
Selenium	mg/kg		

NOTES:

- The sampling technique used to obtain this sample will determine if the sample represents the lot.
- A value of "0" indicates that the value was below detection limits.
-Arsenic and selenium analyses performed by ICP-mass spec at the USU Animal Diagnostic Lab.

-Nitrate-Nitrogen values < 1100 mg/kg - should be safe for all animals.
>1100mg/kg but <2300 mg/kg - use caution; dilute with other feeds; do not feed to pregnant animals.
>2300 mg/kg - DO NOT FEED!

Feed Report

USU Analytical Labs
Utah State University
Logan, Utah 84322-4830
(435) 797-2217
(435) 797-2117 (FAX)

Date Received 10/6/2010
Date Completed 11/2/2010

Name Maureen Kesaano
Address: DR. RYAN DUPONT
1600 CANYON RD
LOGAN UT 84341 CACHE

Lab Number: 1002-5366
Identification: Fresh Duckweed
Feed Material: Duckweed

		AS SAMPLED:	DRY MATTER BASIS:
Moisture	%	93.7	
Dry Matter	%	6.3	100
Protein	%	1.12	17.8
ADF	%	1.15	18.3
NDF	%	1.29	20.5
Unavailable Protein	%		
Ash	%		
Nitrate-Nitrogen	mg/kg		
Total Digestible Nutrients - TDN	%		
Net Energy Lactation - NEL	Mcal/lb		
Net Energy Maintenance - NEM	Mcal/lb		
Net Energy Gain - NEG	Mcal/lb		
MINERALS:			
Calcium	%	0.35	5.54
Phosphorus	%	0.04	0.70
Potassium	%	0.25	3.97
Magnesium	%	0.02	0.34
Sodium	mg/kg	489	7765
Sulfur	%	0.03	0.48
Aluminum	mg/kg	5	84
Boron	mg/kg	30.8	489.0
Cadmium	mg/kg	0.0	0.0
Cobalt	mg/kg	0.0	0.3
Chromium	mg/kg	0.0	0.4
Copper	mg/kg	1.4	21.6
Iron	mg/kg	8.7	138.0
Manganese	mg/kg	12.1	192.0
Molybdendum	mg/kg	0.0	0.0
Nickel	mg/kg	0	1
Lead	mg/kg	0	0
Strontium	mg/kg	7.6	120.0
Zinc	mg/kg	2.5	39.6
Arsenic	mg/kg		
Selenium	mg/kg		

NOTES:

- The sampling technique used to obtain this sample will determine if the sample represents the lot.
- A value of "0" indicates that the value was below detection limits.
-Arsenic and selenium analyses performed by ICP-mass spec at the USU Animal Diagnostic Lab.

-Nitrate-Nitrogen values < 1100 mg/kg - should be safe for all animals.
>1100mg/kg but <2300 mg/kg - use caution; dilute with other feeds; do not feed to pregnant animals.
>2300 mg/kg - DO NOT FEED!

Table A- 1. Degradability of dry matter, neutral detergent fiber and acid detergent fiber of alfalfa hay, corn silage, and duckweed on in vitro fermentation

Treatment	Incubation time, h					
	6	12	24	36	48	96
Dry matter degradability, %						
Alfalfa hay	33.9[a]	44.4[a]	57.3[a]	60.2[a]	65.7[b]	66.8[b]
Corn silage	20.4[c]	28.5[c]	55.8[a]	62.9[a]	71.3[a]	76.2[a]
Duckweed	28.9[b]	37.8[b]	48.7[b]	50.9[b]	54.2[c]	58.6[c]
SEM	0.77	0.88	1.46	1.03	1.02	0.84
Neutral detergent fiber degradability, %						
Alfalfa hay	11.2[b]	16.3[b]	32.6	38.6	45.0	48.5[b]
Corn silage	6.95[b]	10.4[b]	31.1	41.0	49.8	61.3[a]
Duckweed	28.9[a]	32.9[a]	35.5	41.2	43.9	49.3[b]
SEM	2.04	3.68	2.25	1.98	2.44	2.39
Acid detergent fiber degradability, %						
Alfalfa hay	6.00[b]	13.4	28.3	36.2	44.1[a]	46.0[bc]
Corn silage	2.80[b]	11.2	28.4	39.4	49.1[a]	59.0[a]
Duckweed	16.8[a]	24.6	28.2	33.7	35.0[b]	39.9[c]
SEM	2.11	3.79	2.62	1.99	2.33	2.54

SEM = standard error of the treatment means, n = 2.
[a,b,c]Within columns, means without common superscripts significantly different ($P < 0.05$).

Table A- 2. Ruminal fermentation characteristics of alfalfa hay, corn silage, and duckweed on in vitro fermentation

Treatment	Incubation time, h			
	6	12	24	48
Total VFA, mM				
Alfalfa hay	40.6	55.5[a]	68.6[a]	71.6[a]
Corn silage	37.3	49.4[b]	65.1[a]	73.2[a]
Duckweed	38.9	49.8[b]	56.8[b]	60.9[b]
SEM	2.20	1.27	1.93	1.71
Molar proportion of acetate (mol/100 mol)				
Alfalfa hay	68.7[a]	66.1	63.1	64.6
Corn silage	64.5[b]	61.3	56.1	56.6
Duckweed	70.7[a]	65.2	64.8	57.4
SEM	0.75	3.22	2.30	3.96
Molar proportion of propionate (mol/100 mol)				
Alfalfa hay	17.3[a]	17.0	16.7[b]	16.5
Corn silage	16.6[b]	17.0	18.2[a]	18.3
Duckweed	14.9[c]	15.0	14.1[c]	15.3
SEM	0.20	0.90	0.34	0.75
Molar proportion of butyrate (mol/100 mol)				
Alfalfa hay	8.61[b]	9.35	8.64[b]	8.20
Corn silage	12.7[a]	13.3	13.9[a]	13.8
Duckweed	8.41[b]	9.84	9.27[b]	11.5
SEM	0.44	1.25	0.77	1.37
Acetate:propionate				
Alfalfa hay	3.98[b]	3.90	3.79[b]	3.94
Corn silage	3.89[b]	3.62	3.08[b]	3.11

Table A-2. Continued

Duckweed	4.75[a]	4.39	4.59[a]	3.76
SEM	0.078	0.435	0.211	0.39
Ammonia-N (mg/dL)				
Alfalfa hay	26.9	40.5[b]	52.8[a]	56.5[a]
Corn silage	26.1	35.8[b]	43.5[b]	48.3[b]
Duckweed	29.0	46.8[a]	53.7[a]	58.3[a]
SEM	1.17	1.80	0.86	1.51

SEM = standard error of the treatment means, n = 2.
[a,b,c]Within columns, means without common superscripts differ ($P < 0.05$).

Table A- 3. Acid Detergent Lignin results for all treatment combinations considered

Acid Detergent Lignin							
	bag#	bag wt	input	DM input	po ADL	%ADL	ave. %ADL
alfalfa hay	31	0.4736	0.5228	0.4962	0.5065	6.72	7.48
	32	0.4886	0.5435	0.5159	0.5270	7.54	
	33	0.4861	0.5167	0.4905	0.5258	8.19	
corn silage	68	0.4865	0.5176	0.4989	0.5050	3.80	3.93
	69	0.4822	0.5157	0.4970	0.5016	4.00	
	70	0.4904	0.5098	0.4913	0.5095	3.99	
duckweed	817	0.4925	0.3204	0.3181	0.5072	4.77	4.81
	818	0.4970	0.3197	0.3174	0.5102	4.31	
	819	0.4980	0.3335	0.3311	0.5152	5.34	
dw-ah	820	0.4758	0.3144	0.2974	0.4927	5.84	5.65
	821	0.4877	0.3156	0.2985	0.5061	6.33	
	822	0.4886	0.3144	0.2974	0.5023		
dw-cs	823	0.4878	0.3557	0.3386	0.5021	4.37	4.18
	824	0.4661	0.3201	0.3047	0.4780	4.06	
	825	0.4796	0.3245	0.3089	0.4918	4.10	
dw-ah-cs	826	0.4919	0.3623	0.3428	0.5114	5.83	5.69
	827	0.4892	0.3334	0.3155	0.5056	5.35	
	828	0.4925	0.3321	0.3142	0.5105	5.88	
Blank	829	0.5055	0	0	0.5050	0.9990	

Table A- 4. Ethanol yield (%, v/v) from dry duckweed biomass fermentation

Run		ethanol yield, % V/V							theoretical %, v/v	starch, %
#	Date	1	2	3	4	5	6	7		
1	10/30/2009	0.14	0.26	0.35	0.3	0.19	0.25	0.25		
2	06/28/2010	0.03	0.06	0.06	0.12				0.15	1.80
3	07/13/2010	0.03	0.07	ND	ND	ND				
4	08/10/2010	ND	ND	0.06	0.06	0.07	0.06		0.10	1.53
5	10/27/2010	0.22	0.22	0.22	0.21	0.23	0.24	0.26	0.32	4.70

Table A- 5. Ethanol yield (%, v/v) from fresh duckweed biomass fermentation

Run	Date	ethanol yield, % v/v							theoretical, % v/v	starch, %
#		1	2	3	4	5	6	7		
1	10/30/2009	0.53	0.56	0.58	0.69	0.63	0.59	0.65		
2	06/14/2010	0.04	0.01	ND	0.17	0.56	-	-		
3	06/23/2010	0.04	0.03	0.06	0.09	0.4	-	-	0.486	28.37
4	08/3/2010	0.03	0.15	0.16	0.17	0.16	0.16	-	0.192	11.00
5	10/27/2010	0.15	0.24	0.30	0.35	0.43	0.49	0.5	0.62	16.4

Table A- 6. Comparison of feed quality of the digested solids, fresh and dried duckweed biomass

	Fresh DW	Air dried DW	Oven dried DW	Digested dry DW	Digested fresh DW
Crude protein	25.03	21.50	20.90	15.40	12.50
NDF	28.42	24.50	33.90	43.20	40.70
ADF	18.95	22.90	23.10	36.20	28.90
RFV	242.69	269.81	194.57	130.71	151.73

Table A- 7. P measurement using the modified lab procedure

Sample	Description	Tare, g	Tare+ sample, G	Sample wt., mg	Dilution	ABS@ 890nm @12 min.	[P] (mg/L)	mg-P/mg-tissue	**%P**
1	R1 01-05 Nov			185.1	500	0.257	150.22	0.008	**0.81**
2	R2 01-05 Nov			131.3	500	0.16	92.96	0.007	**0.71**
3	R2 18-22 Oct			161	500	0.242	141.36	0.009	**0.88**
4	R1 10-15 Oct			178	500	0.227	132.51	0.007	**0.74**
5	R2 15-19 Oct			152	500	0.24	140.18	0.009	**0.92**
6	R1 15-20 Oct			135	500	0.183	106.54	0.008	**0.79**
7	R1 04-08 Oct			171.4	500	0.201	117.16	0.007	**0.68**
8	R1 18-22 Oct			182.1	500	0.247	144.32	0.008	**0.79**
9	R1 25-29 Oct			192.5	500	0.278	162.61	0.008	**0.84**
10	R2 25-29 Oct			134.2	500	0.206	120.12	0.009	**0.90**
A	Acid blank				500	0.007	2.66		
B	grape petiole			197.7	500	0.107	61.68	0.003	**0.31**
C	Grape petiole	14.8921	15.0277	135.6	500	0.087	49.88	0.004	**0.37**
D	Grape petiole	15.935	16.0388	103.8	500	0.069	39.25	0.004	**0.38**

APPENDIX B: SAMPLE CALCULATIONS

Theoretical ethanol yield

Using factors obtained from equations 1 and 2

Fresh duckweed (3200 g) wet basis, moisture content 95.67 %, and starch 16.4 %

Amount of duckweed (dry Weight) = 3200 g X 0.0433 = 138.56 g

Starch content = 138.56 g X 0.164 = 22.72 g

Glucose produced using 1.11 conversion factor = 22.72 X 1.11 = 25.22 g

Theoretical ethanol yield assuming 0.511 conversion factor = 25.22 X 0.511= 12.89 g

Volume of ethanol = $\frac{12.89\ g}{0.79\ g/ml} = 16.32\ ml$

Yield in %, v/v = $\frac{16.32\ ml}{2625\ ml}\ x\ 100 = 0.62\%$

Or $\frac{12.89\ g\ Ethanol * 1000\ mg/g}{128.56\ g\ DW} = \frac{100.3 mg}{g\ DW}$

Anaerobic digestion % VS reduction

$$\%\ vs\ reduction = \frac{VS_{feed} - VS_{sludge}}{VS_{feed}}\ x\ 100$$

$$= \frac{5181.469\ mg/l - 3220.18\ mg/l}{5181.469}\ x\ 100 = 38\%$$

Sample calculation for RFV using In vitro fermentation results

$$\text{Relative Feed Value} = \frac{(\text{intake potential} * \text{Digestible DM})}{\text{constant}}$$

$$\text{intake potential} = \frac{120}{\text{NDF}}$$

$$\text{Digestible D M} = 88.9 - (0.779 * \text{ADF}) \quad \text{and constant} = 1.29$$

$$\text{RF V} = \frac{\left({}^{120}/_{30.2}\right) * [88.9 - (0.779 * 13.7)]}{1.29} = 240.7$$

Sample calculation for RFV using feed analysis report

$$\text{Relative Feed Value} = \frac{(\text{intake potential} * \text{Digestible DM})}{\text{constant}}$$

$$\text{intake potential} = \frac{120}{\text{NDF}}$$

$$\text{Digestible D M} = 88.9 - (0.779 * \text{ADF}) \quad \text{and constant} = 1.29$$

$$\text{RF V} = \frac{\left({}^{120}/_{29.95}\right) * [88.9 - (0.779 * 20.43)]}{1.29} = 226.7$$

APPENDIX C: REFERENCE TABLES

Table C- 1. Utah feed values for alfalfa hay courtesy of USDA-Dept of Ag

	RFV	ADF	CP	NDF	TDN-100%	TDN-90%	CP
Supreme	> 185	< 27	< 22	< 34	> 62	> 55.9	> 22
Premium	170 - 185	.27 - .29	20 - 22	34 - 36	60.5 – 62	54.5 - 55.9	20 – 22
Good	150 - 170	.29 - .32	18 - 20	36 - 40	58 – 60	52.5 - 54.5	18 – 20
Fair	130 - 150	.32 - .35	16 - 18	40 - 44	56 – 58	50.5 - 52.5	16 – 18
Utility	< 130	> . 35	> 16	> 44	< 56	< 50.5	< 16

Table C- 2: NRC nutrient requirements for beef cattle

Vitamin and mineral requirements and maximum tolerable concentrations [ab]					
	Requirements				
	unit	Gestation	Lactation	Max. Tolerable concentration	Study values
Vitamins required by beef cattle					
A	IU/kg	2800.0	3900.0	----	
D	IU/kg	275.0	275.0	----	
Minerals required by beef cattle					
Calcium	%	see tables 1-4	see tables 1-4	2.0	4.0 – 7.0
Chlorine	%	----	----	----	----
chromium[c]	mg/kg	----	----	1000.0	1.0 – 5.0
Cobalt	mg/kg	0.1	0.1	10.0	0.0 – 0.5
Copper	mg/kg	10.0	10.0	100.0	6.0 – 67.0
Iodine	mg/kg	0.5	0.5	50.0	----
Iron	mg/kg	50.0	50.0	1000.0	233.0 –1084.0
Magnesium	%	0.1	0.2	0.4	0.4 – 0.6
Manganese	mg/kg	40.0	40.0	1000.0	155.0 – 337.0
Molybdenum c	mg/kg	----	----	5.0	BDL
Nickel c	mg/kg	----	----	50.0	1.0 – 23.0
Phosphorus	%	see tables 1-4	see tables 1-4	1.0	0.8 – 1.0
Potassium	%	0.6	0.7	3.0	3.0 – 5.0
Selenium	mg/kg	0.1	0.1	2.0	----
Sodium	%	0.07[d]	0.1	----	4887.0 -11874.0 mg/kg
Sulfur	%	0.2	0.2	0.4	0.6 – 0.8
Zinc	mg/kg	30.0	30.0	500.0	12.0 – 176.0
Minerals toxic to beef cattle					
Aluminum	mg/kg	----	----	1000.0	209.0 – 2591.0
Arsenic	mg/kg	----	----	50.0[e]	----
Bromide	mg/kg	----	----	200.0	----
Cadmium	mg/kg	----	----	0.5	0.1 – 0.5
Fluorine	mg/kg	----	----	40.0[f]	----
Lead	mg/kg	----	----	30.0	0.0 – 7.0
Mercury	mg/kg	----	----	2.0	----
Strontium	mg/kg	----	----	2000.0	211.0 – 243.0

[a] Adapted from NRC (1996),
[b] Concentrations are expressed on a dry matter basis; NRC (1996),
[c] Evidence exists to indicate that there is a dietary requirement for this element. Data is not extensive enough to establish specific dietary concentration.
[d] Given as a range between 0.06 - 0.08% of diet dry matter.
[e] Organic forms of arsenic can have maximal tolerable concentrations of 100mg/kg.
[f] Given as a range of 40 - 100 mg/kg of diet dry matter. BDL-Below detection limit

APPENDIX D-MODIFIED P METHOD

Plant tissue Digestion Procedure by Jon Farrell, grad student Utah State University (June 22 2010)

Adapted from dry ash and wet ash tissue digestion procedures with aqua regia soln. and standardized against grape petiole leaves (0.38%-P) from NAPT guidelines.

YASH P. KALRA, HANDBOOK OF REFERENCE METHODS FOR PLANT ANALYSIS, CRC PRESS, 1998.

1. Measure out 120-200mg*(dried/ground) duckweed into 10mL glass vial(s).
2. Ash plant tissue by heating vial(s) un-capped in 550ºC muffle furnace for at least 30 minutes. Remove and let cool.
3. Prepare *aqua regia* solution with 6:3:1 (DI H_20: conc. HCl: conc. HNO_3).
4. Slowly pipette 10mL *aqua regia* soln. into each vial with ashed plant tissue. Mix well and allow to sit for approx. 15 min.
5. Dilute appropriately.
6. Prepare an acid blank.
7. Measure for %P using one of the following two methods (A or B):

Measurement for Phosphorus content:

A. Using Ion Chromatography
    i. Dilute digested duckweed 1:50 in another 10mL vial.
        1. Neutralize by adding 1mL of 1M NaOH to 8.8 mL DI H_20.
        2. Pipet in 200uL of digested solution from step #4.
        3. Mix.
    ii. Measure using IC method "anion_JF_v1.met"
    iii. Quantify using the following formula:
        1. [P conc. (mg/L)/100] / [plant mass (mg)/100] * 100 = %-P
B. Using 890nm spectrophotometer (adapted from Joan's P-lab handout)

Adapted from Standard Methods (APHA) 4500-P. E Ascorbic Acid Method

i. Prepare Combined reagent and Mix in the following order (stable for 4 hours):
    1. 50mL-5N H_2SO_4 (Dillute 70mL conc. Sulfuric acid in 500mL DI water)

2. 5mL-Potassium antimonyl tartrate soln. (Dissolve 1.3715 g potassium antimonyl tartrate in 400mL distilled water in a 500mL volumetric flask and dilute to volume—not x4H_2O's)
3. 15mL-Ammonium molybdate solution (Dissolve 20g ammonium molybdate in 500 mL distilled water. Store in a glass-stoppered bottle)
4. 30mL-Ascorbic acid, 0.1M (Dissolve 1.76g ascorbic acid in 100mL distilled water. The solution is stable for about 1 week @ 4ºC—throw out when it turns yellow)

ii. Dilute sample(s) accordingly; method detection range is from approx. 25ug/L to 500ug/L
    1. 1:10=approx. 1-2.5mg(PO_4-P)/L range; use 1mL sample/10mL
    2. 1:25=approx. 1.25-6.2mg(PO_4-P)/L range; use 400uL sample/10mL
    3. 1:50=approx. 2.5-12.5mg/L range; use 200uL sample/10mL
    4. For solids (100-200mg) use 1:500 for approx. 0.5-2%-P; dilute once with 1mL sample/10mL and then dilute again at 200uL/10mL
iii. Prepare at least 3 standards (i.e. 25ug/L, 250ug/L, and 500ug/L)
iv. Add 1.6mL combined reagent to sample and let stand for 12 minutes.
v. Measure @890nm

*120-200mg-plant tissue assumes approximately 1%-P and is further diluted 1:50 (for IC analysis) or 1:500 (for colorimetric analysis)

APPENDIX E-ANAEROBIC DIGESTION RESULTS

Format of tables as presented in the spreadsheet (see CD attached)

Table E-1: Total solids results

Results for Total solids for fresh fed (R1) and dry fed (R2) digesters, Sep 2009 - Nov 2010									
Date	Reactor	Initial Wt (g)	Final Wt (g)	Beaker Wt (g)	Δ Wt (g)	Solids Wt (g)	Solids Wt (mg)	Vol (L)	[TS] mg/L

Table E-2: Volatile solids results

Results for Votal solids for fresh fed (R1) and dry fed (R2) digesters, Sep 2009 - Nov 2010								
Date	Reactor	Vol (ml)	initial wt (g)	Final wt (g)	ΔWt (g)	solids wt (mg)	Vol (L)	VS (mg/L)

Table E-3: Gas volume and composition results

Results for gas volume and composition for fresh fed (R1) and dry fed (R2) digesters, Sep 2009 - Nov 2010												
Date	Reactor	pH	Temp, ^{o}C	Pressure mmHg	gas yield, ml	gas yield @ stp	CH_4 (ml)	CO_2 (ml)	O_2 (ml)	% CH_4	% O_2	% CO_2